ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *BREVICEPS*

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Thesis submitted to the Faculty of Science,
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in fulfilment of the requirements for the
degree of Doctor of Philosophy.

JOHANNESBURG
October 1998

ABSTRACT

By focusing on the mate recognition system, this study succeeds in resolving a number of taxonomic difficulties and uncertainties in the genus *Breviceps* and uncovers an undescribed cryptic species.

Spectrographic analysis of advertisement calls collected from 62 *Breviceps* populations in Mozambique, Zimbabwe, Swaziland and eastern South Africa reveals the presence of six separate groups, each characterised by distinct temporal call characters. It is argued that these groups represent genetical species as defined by the recognition concept of species (sensu Paterson, 1985).

Significant differences between the advertisement calls of these six *Breviceps* species include the presence or absence of amplitude modulation, duration, number of pulses, the grouping of calls within the call bout, and in one case, dominant frequency. In contrast to many other genera of frogs, pulse rate is not highly differentiated among the species of *Breviceps* sampled.

B. adspersus and *B. mossambicus* give short, pulsed calls which differ in duration, number of pulses and the grouping of calls within the call bout. *B.a. pentheri* is retained as a valid taxon, but its distribution is limited to the Eastern Cape Province. Populations from the eastern escarpment of South Africa, which were previously referred to *B.a. pentheri*, are shown to be conspecific with coastal populations of *B. mossambicus* in Kwazulu/Natal. The call data do not support the suggestion (in litt.) that extensive hybridisation occurs between *B. adspersus* and *B. mossambicus*.

B. verrucosus and *B. sylvestris* give longer, pulsed calls which are not grouped, and differ from one another in call repetition rate and, to a lesser extent, duration and number of pulses. Retention of the taxa *B.v. verrucosus* and *B.v. tympanifer* is not supported.

Two species give unpulsed calls, viz: *B. poweri*, which emits short, grouped calls and an undescribed species which gives long, high-pitched calls.

Morphological analysis of the material collected during this study shows that certain characters have lower diagnostic value than was previously accorded to them. For example, dorsal markings and the relative length of the outer finger do not permit the separation of *B. adspersus*, from *B. mossambicus* or *B. sp.*, and recognition of the subspecies *B. v. tympanifer* on the basis of colour and dorsal markings is not supported.

Population localities, distribution maps, and data pertaining to other aspects of the reproductive biology of the species studied, such as call site, chorus structure and nest construction, are included in the appendices.

I declare that this thesis is my own, unaided work unless specifically acknowledged in the text. It has not been submitted before for any degree or examination in any other university.

Leslie Rory Minter

29th October, 1998.

Everything existing in the Universe is the fruit of chance and of necessity.

Democritus

PREFACE

Despite the widespread occurrence of *Breviceps* in southern Africa, little is known about the biology of the various species. Their cryptic colouration, slow movements and fossorial habits render them inconspicuous, and a sharp eye and a great deal of patience are necessary in order to locate a calling male in the field. Amplexing pairs are seldom found, as oviposition takes place underground.

The subject of this study is the reproductive biology of several species of *Breviceps*, focusing mainly on the structure of the advertisement call. Until now, the criterion of morphological similarity has been used to delineate species within this genus. Advertisement call structure is, however, a more reliable indicator of the existence of a common gene pool since it is primarily responsible for mate recognition in frogs and is species specific. The main objectives of this study are to confirm the status of described *Breviceps* species in the areas sampled, determine whether widespread hybridization occurs between *B. adspersus* and *B. mossambicus* (as reported in the literature), and identify any undescribed *Breviceps* species which may be present.

Advertisement calls were recorded in spring, when choruses develop during and immediately following the first rains. The collection of the data was hampered by a shift in rainfall patterns and a prolonged drought which made it difficult to plan field trips in advance. In addition, many collecting trips were relatively unsuccessful due to the inaccuracy of weather reports or because insufficient rain had fallen to stimulate the development of a strong chorus. Consequently the samples from many localities are relatively small.

Nevertheless, it was found that under favourable conditions it is possible to obtain sufficient specimens and adequate field data. Also, specimens are relatively easy to keep in captivity and amplexing pairs will breed if offered a suitable substrate. The opportunity therefore exists for further investigation of the biology of this interesting and little-known genus of terrestrial-breeding frogs.

Acknowledgements

I would like to acknowledge the support and encouragement of my supervisor, Professor Neville Passmore. His enthusiasm and patience over the extended period taken to complete this study and his guidance in both practical and theoretical matters were invaluable.

In the course of the fieldwork I received the assistance and hospitality of many people, to whom I am deeply grateful. These include Toy Bodbijl, Phil Bishop, Don Broadley, Marius Burger, Richard Boycott, Bill Branch, Buster Culverwell, Ken Gamble, Ian Garland, the late Amy Jacot-Guillarmod, Mark Harman, Manuel Joachim, Scotty Kyle, Dinis Lissave, Miguel Massunda, Ed Ostroski, Sean Paterson and Jerry Theron. In particular I would like to thank my friend and companion on many field trips, Rupert Harris, for his able assistance, culinary skill, and stimulating conversations.

I thank the following people for information, specimens and discussions of various theoretical aspects of this study: Pat Backwell, Phil Bishop, Bill Branch, Don Broadley, Alan Channing, Mandy Dyson, Niels Jacobsen, Angelo Lambiris, Hugh Paterson, John Poynton, Rob Toms and Eddie van Dijk.

The following institutions are thanked for permission to work in the areas under their jurisdiction: the Cape Department of Nature Conservation; the Natal Parks Board; the Department of Tourism and the Environment, Kwazulu/Natal; and the Department of Forestry and Wildlife, Mozambique.

I acknowledge with gratitude, the financial assistance received from the Communication Biology Research Group, Zoology Department, University of the Witwatersrand, which provided most of the funding for this study, the Twinstreams Fund which enabled me to attend the 2nd World Congress of Herpetology in Adelaide, and the University of the North for a post-graduate grant and for allowing me to spend long periods in the field.

Lastly, I thank my wife Jean for her patience and encouragement and for coping with numerous domestic emergencies which inevitably arose while I was away in the field.

Animal ethics clearance

Clearance for the collection and killing of specimens was obtained from the Animal Ethics Screening Committee, University of the Witwatersrand (AESC No. 89/95/1).

Publications

Minter, L.R. 1995. The advertisement call and other aspects of the reproductive biology of *Breviceps adspersus* Peters (Anura, Microhylidae). Madoqua 19(1): 37-44.

Minter, L.R. 1997. Advertisement call structure and morphology of *Breviceps mossambicus* Peters and *B. poweri* Parker (Anura: Microhylidae) from northern Mozambique. Ann. Natal Mus. 38: 5-19.

ABBREVIATIONS USED IN THE TEXT

°C	degrees centigrade
cm	centimetre
CI	confidence interval
C.int.	call interval
dB	decibel
E	east
Fig.	figure
g	gram
Hz	Hertz
kHz	kilohertz
km	kilometer
Loc.	locality
m	slope of regression line
Max	maximum
Min	minimum
min	minute
ms	millisecond
N	sample size
NB	narrow band spectrogram
r	correlation co-efficient
s	second
SD	standard deviation
SMRS	specific mate recognition system
SPL	sound pressure level
SVL	snout-vent length
WB	wide band spectrogram

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INTRODUCTION

1. The genus *Breviceps*

1.1 Biology

This genus comprises 13 described species of entirely terrestrial, globose, fossorial frogs which are endemic to sub-Saharan Africa. The species occupy a wide range of habitats including forest, montane grassland, open and closed woodland and vegetated coastal dunes. Despite their wide distribution and abundance, even in urban areas, relatively little is known of their biology. This is largely due to the fact that they spend most of the year underground and when on the surface are rendered inconspicuous by their slow movements and cryptic colouration. During the breeding season, males often use well concealed call sites. The call has a ventriloqual quality and as a consequence, calling males are extremely difficult to locate. An inexperienced person may spend several hours trying to locate a single individual, often without success: this has led to the erroneous belief that they call from underground or from the mouths of deep burrows into which they retreat when disturbed.

These frogs walk rather than hop, and use the enlarged, spade-like metatarsal tubercles on their feet to burrow rapidly, backwards, into the soil. During dry periods they remain below the surface, sometimes in groups (Jacobsen, 1989; Milstein, 1967), and some species secrete a mucous cocoon (Channing pers. comm.). When alarmed they inflate their lungs, secrete a sticky, white substance from the skin on the dorsal surface of the body (Fig. 1b) and sometimes emit a piercing alarm call.

Breviceps emerge after rain to feed on ants, termites, beetles, moths, woodlice, amphipods, juvenile millipedes, caterpillars and other small arthropods (Barbour and Loveridge, 1928; Loveridge, 1925; Fitzsimons, 1935, 1958; Wager, 1965; Poynton and Pritchard, 1976; Channing & van Wyk, 1987). Poynton & Pritchard (1976) analysed the stomach contents of *B. adspersus* and *B. verrucosus* and found that the former contained large numbers of alate termites and worker ants, while the latter

contained invertebrates characteristic of the forest floor. Animals preying on *Breviceps* include the black-backed jackal (Botma, 1971; Viljoen and Davis, 1973), bush-pig (Palmer, 1982), fiscal shrike (Hewitt and Power, 1913), olive thrush (Oatley, 1970), hadeda ibis (Wager, 1957, 1965), vine snake and white-lipped snake (Loveridge, 1953), night adder (Blake, 1965), and Mozambique spitting cobra (Broadley, 1971).

Reproduction occurs during the rainy season. Choruses usually develop immediately after the first heavy rains except in the winter rainfall region, where breeding may be delayed by low temperatures. Chorus structure has been briefly described by Ranger (in Hewitt, 1937:117), Wager (1965:55), and Poynton and Pritchard (1976) but has never been thoroughly studied. Males have been seen calling from the surface, elevated positions on vegetation, shallow depressions, the mouths of shallow or deep burrows, and have been reported to call from below the surface (Wager, 1965; Pickersgill, 1975; Poynton and Pritchard, 1976; Lambiris 1989a & b). In continuous rain or drizzle, males may call throughout the day as well as at night.

During amplexus the male and female adhere to one another (Fig. 1a) by means of a sticky white secretion (Fig. 1b) produced by dermal glands on the dorsal and ventral surfaces (Wager, 1960). The histology of these glands has been described by Visser et al. (1982).

Descriptions of the egg masses of several species can be found in Barbour and Loveridge (1928), Fitzsimons and van Dam (1929), Gow (1962), de Villiers (1988), Wager (1965), Rose (1962) and Visser (1979). In these species, eggs are laid in batches of 20 - 56 in chambers below the surface of the soil, rocks or fallen logs. The egg masses are covered by a layer of eggless capsules (Fig. 1c). The larval development of one species has been described by de Villiers (1929), Fitzsimons and van Dam (1929), and Swanepoel (1970). As development proceeds, the tadpoles break out of the egg capsule and wriggle around actively, working the remaining jelly and liquid into a froth. The female remains close to the egg chamber until development is complete, 6 - 8 weeks after the eggs are laid, but the reason for her presence is not known.

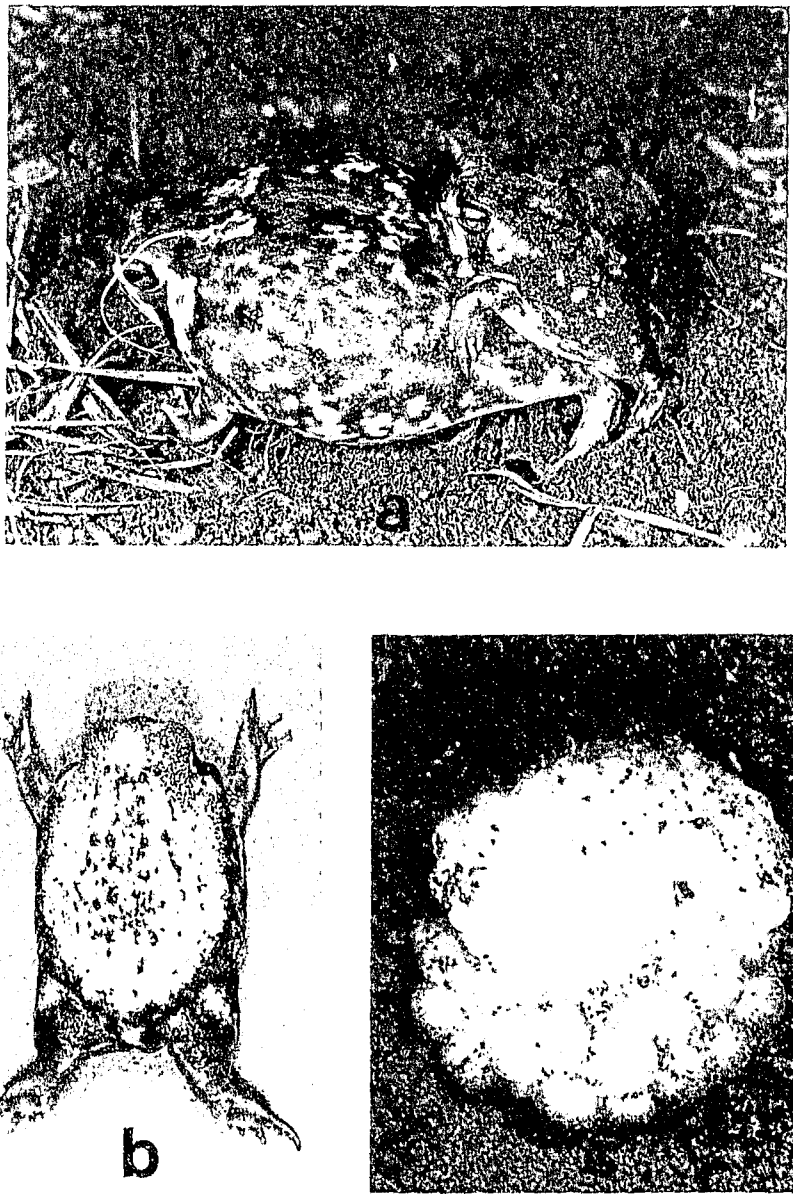

FIGURE 1. *Breviceps a. adspersus*: (a) amplexing pair; (b) secretion on dorsal surface of male; (c) egg mass in subterranean chamber.

1.2 Introduction to the taxonomy of *Breviceps*

In 1758 Linnaeus gave the name *Rana gibbosa* to a species of frog that had been recorded from the Cape by Seba in 1735. This was, according to Parker (1934), the first description of a species belonging to the Microhylidae. In 1820 Merrem transferred *Rana gibbosa* to a new genus, *Breviceps*, under the name *Breviceps gibbosus*. Between 1842 and 1980, 21 species and 14 subspecies were added to the genus by Rapp (1842), Peters (1854, 1882), Werner (1899, 1903), Boulenger (1907), Hewitt (1925, 1932), Power (1926), FitzSimons (1930, 1946, 1947, 1950), Parker (1934), Hoffman (1940, 1944), Pienaar (1963), Poynton (1963, 1964, 1980), and Broadley (1971). Taxonomic revisions of the genus as a whole by Boulenger (1910), Hewitt (1911), Power (1926), Parker (1934), Poynton (1964), and surveys of species occurring in specific geographical areas by Stewart (1967), Broadley (1971), Channing and van Dijk (1976), Pienaar et al. (1976), Passmore and Carruthers (1995), Poynton and Broadley (1985), Lambiris (1989a & b), Jacobsen (1989), Channing and Griffin (1993), resulted in a number of these names falling into synonymy. In addition to the recognition of taxa at the species and subspecies levels, there have been reports of extensive hybridization between *B. adspersus*, *B. mossambicus* and *B. poweri* resulting in the presence of hybrid swarms in a broad zone stretching from Zululand northwards through southern Mozambique and eastern Zimbabwe to Malawi (Poynton, 1964, 1982; Stewart, 1967; Poynton and Broadley, 1985; Lambiris, 1989a).

Thirteen species and eight subspecies of *Breviceps* are currently recognised, but differences of opinion still exist among herpetologists regarding the status of some of these taxa, eg. *B. adspersus* Peters, *B. adspersus pentheri* Werner, *B. mossambicus* Peters, *B. poweri* Parker, *B. maculatus* FitzSimons and *B. rosei vansoni* FitzSimons.

1.3 Taxonomic methodology

The taxonomic treatment of this genus has been based entirely on morphological characters. As more material accumulated, certain of these characters were shown to be of no diagnostic value (eg. exposed/hidden tympanum in *B. verrucosus* Rapp, ven-

tral surface immaculate/mottled in *B.montanus* Power, gular patch divided/ undivided in *B. adspersus*) as the samples upon which the earlier descriptions had been based did not adequately reflect the range of variation actually present. Furthermore, characters which had been used to separate species were found to be shared by those species. As Poynton (1964:70) noted, "the diagnosis of the different forms of *Breviceps* is particularly difficult on account of the variation and slight morphological differentiation". In more recent revisions, the genetical, biological species (isolation) concept was embraced. For example, Poynton (1964:11) stated: "In this treatment, the measure of a species is taken to be its degree of reproductive isolation, rather than its degree of morphological differentiation".

In his monographic revision of the South African Anura, Poynton (1964:5) noted that "The most distinctive and diagnostic feature about any amphibian species is its call." Subsequent studies of the advertisement calls of South African frogs led to the discovery of cryptic species of *Tomopterna* (Passmore & Carruthers, 1975) and *Heleophryne* (Boycott, 1982) and have helped to resolve a taxonomic problem in the genus *Pyxicephalus* (Channing et al., 1994). Referring to *Breviceps adspersus* and *B. mossambicus*, Poynton (1964:80) notes that "the distinctness of the two forms is placed beyond doubt by a clear difference in the pitch of the call." Lambiris (1989a&b) gave aural descriptions of *Breviceps* advertisement calls, while Passmore and Carruthers (1995) published sonagrams of the calls of six species, and synonymised *B. maculatus* with *B. verrucosus* on the basis (partly) of the similarity of single samples of their advertisement calls. Lambiris (1989a:65) challenged this decision with an unsupported statement that "there is extensive intraspecific variation in calls within the genus". Up to the present time, however, no-one has undertaken a detailed analysis of the advertisement calls of any *Breviceps* species.

Thus, in the absence of detailed behavioural, ecological, biochemical and other data, taxonomists have had to adopt a "pragmatic approach" in which a species is characterised by a unique set of morphological characters which constitutes its diagnosis. Such species are regarded as an "hypothesis about the presence of a reproductively inclusive (and perhaps exclusive) group of amphibians" (Poynton and Broadley,

1985:503). It is this hypothesis which is being tested in the present study.

1.4 General distribution.

Breviceps occurs from the Cape peninsula northwards to Angola, southern Zaire and Tanzania. A higher concentration of species occurs in the south: ten are endemic to South Africa, two have a wider distribution, and one is restricted to an area north of the Zambezi (see Poynton, 1964 and Passmore and Carruthers, 1995 for distribution maps). Existing distribution records for some taxa are of little value because of the uncertainty regarding their taxonomic status and the absence of reliable diagnostic characters (van Dijk, 1971). The existing distribution data have however been used to defend the status of species and subspecies, to infer the identity of specimens, and in ecological and zoogeographic analyses (eg. Inger, 1959; Poynton, 1982; van Dijk, 1977, 1982).

2. The taxonomy and distribution of species dealt with in this study

2.1 *Breviceps adspersus* Peters 1882

2.1.1 Taxonomy

The failure to identify reliable diagnostic morphological characters in *B. adspersus*, *B. mossambicus*, *B. poweri* and *B. adspersus pentheri* (formerly *B. pentheri*) has resulted in considerable taxonomic confusion, as the following account shows.

***B. adspersus adspersus* Peters 1882** (Fig. 2 a&b)

Peter's (1882) short description of *B. adspersus* was based on specimens from Damaraland and the Transvaal between 25° and 26°S. The Transvaal locality has been pinpointed by Poynton & Broadley (1985) as Botsabelo Mission (established 1865), which is located about 8km north of Middelburg, Mpumalanga. *B. adspersus* was placed in the synonymy of *B. parvus* Hewitt (an Eastern Cape species) by Power (1926) but was resurrected by Parker (1934). Fitzsimons (1930), described *B. pretoriensis* on the basis of a single female specimen from Jericho near Pretoria, but this

was later placed in the synonymy of *B. adspersus* by Parker (1934). Poynton (1964) suggested that this specimen might form part of a morphological cline along which individuals become more robust towards the west. Because many specimens possess a mixture of the characters considered to be diagnostic for *B. adspersus* and *B. mossambicus* the status of *B. adspersus* has often been questioned: Fitzsimons (1939) suggested that *B. adspersus* be placed in the synonymy of *B. mossambicus*, while Broadley (1966, 1971), Pienaar et al. (1976), and Poynton (1980) placed *B. adspersus* in the synonymy of *B. mossambicus* as a subspecies, *B. mossambicus adspersus*. In recent taxonomic papers (Poynton, 1982; Poynton & Broadley, 1985; Lambiris, 1989a) these two taxa have been treated as species, while specimens possessing intermediate diagnostic character states have been interpreted as hybrids. Hybridization between *B. adspersus* and an undescribed Angolan species has also been reported by Poynton & Broadley (1985). Following Poynton's (1964) inclusion of *B. pentheri* within this species as *B.a. pentheri*, *B.a. adspersus* became the nominate subspecies.

***B. adspersus pentheri* Werner 1899.** (Fig. 2 c&d)

Werner (1899) described *B. pentheri* on the basis of a 15mm specimen from the Eastern Cape, "apparently Grahamstown". The type specimen was covered in a light brown layer of "excrement", which Werner took to indicate that it had burrowed into the faeces of a ruminant! A more likely explanation is that it was merely covered by particles of sand and humus which had adhered to its sticky secretions released during its capture. One wonders how many ardent herpetologists subsequently searched industriously through piles of ruminant faeces, in pursuit of the "elusive" *B. pentheri*!

In 1910, Boulenger placed *B. pentheri* in the synonymy of *B. gibbosus* L. while recognising *B. adspersus* as a valid species. In 1926 Power synonymised both *B. pentheri* and *B. adspersus* with *B. parvus* Hewitt 1925. Parker (1934) resurrected *B. pentheri* and placed *B. parvus* Hewitt and *B. parvus caffer* Hewitt (1932), from the Eastern Cape, in its synonymy as *B. pentheri pentheri* and *B. pentheri caffer* respectively. He remarked however, that *pentheri* was probably only a southern form of *adspersus*. Poynton (1964) endorsed this view by placing *pentheri* in the synonymy of *B. adspersus* as a subspecies, *B. adspersus pentheri*; after examining additional material from

Grahamstown he also felt that there was no justification for retaining Hewitt's subspecies *caffer*.

2.1.2 Distribution

Parker (1934) gave the distribution of *B. adspersus* as "N.E. Cape Province, Natal and Zululand, Transvaal, Zimbabwe, Botswana and Namibia". Poynton (1964) increased the range to include Swaziland and Mozambique, and restricted its distribution in KwaZulu/Natal to areas below about 4000 feet above sea level. Poynton (1982), and Poynton & Broadley (1985) restricted "pure" *adspersus* to "Natal (except the Mozambique Plain), Transvaal (except the eastern edge), Botswana, Namibia, and the Livingstone area of Zambia", and suggested that north of these areas, i.e. "northern Natal, southern Mozambique, Zimbabwe, eastern Botswana, eastern Transvaal and Malawi", *B. adspersus* hybridises extensively with *B. mossambicus*. Lambiris (1989a) extended the distribution of "pure" *adspersus* slightly to include the Northern Cape and the Free State, and restricted its distribution in KwaZulu/Natal to areas below about 1000m.

B. a. pentheri has been recorded in the Eastern Cape from Port Elizabeth inland to Grahamstown, King William's Town and Kei Road, and along the eastern uplands and great escarpment of the Transkei, KwaZulu/Natal and the Transvaal (Poynton, 1964, 1982; Lambiris, 1989a). Poynton (1964) noted that *B.a. pentheri* "appears to lie on the fringe of *adspersus* on the upper eastern plateau slopes, the line of division apparently coinciding with the 13°C mean July isotherm in Natal".

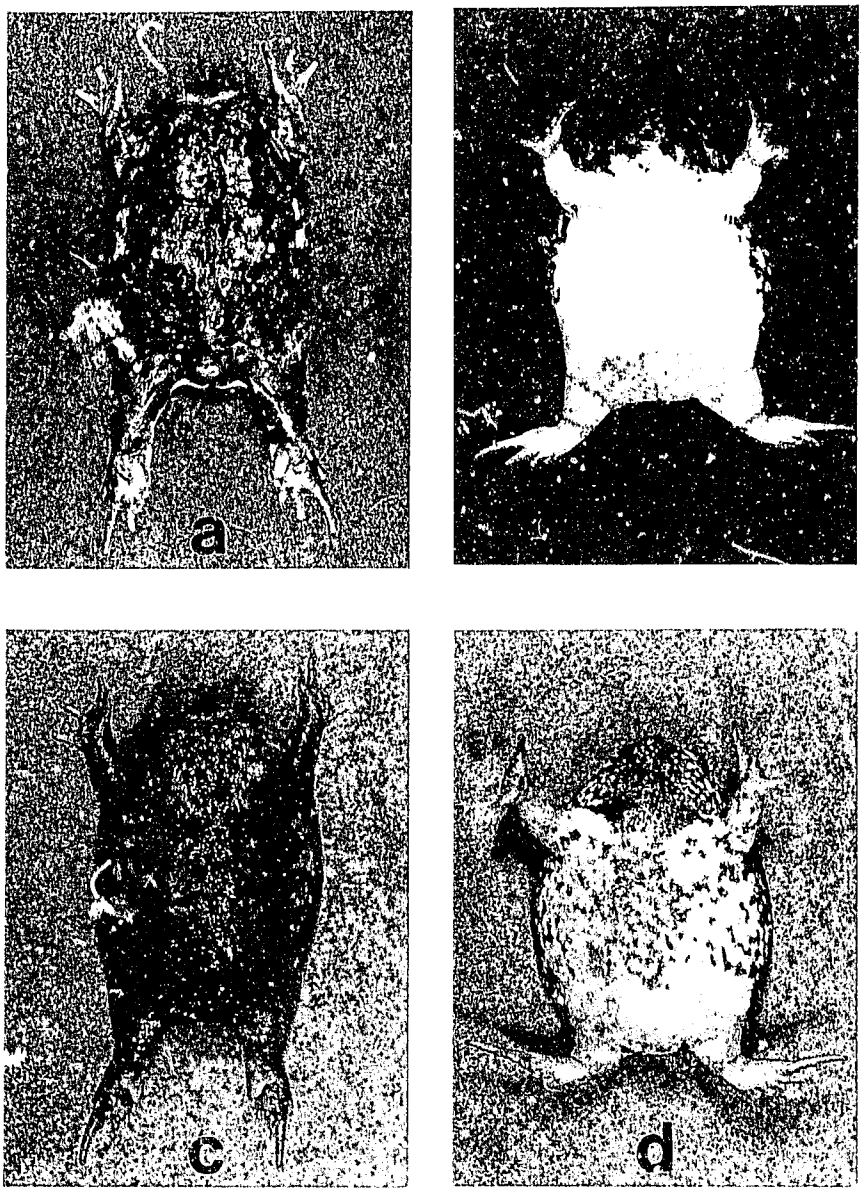

FIGURE 2. (a) *B.a. adspersus* male from Loc.42 (Pietersburg), dorsal; (b) ditto, ventral; (c) *B.a. pentheri* male from Loc.7 (Grahamstown), dorsal; (d) ditto, ventral.

2.2 *Breviceps mossambicus* Peters 1854. (Fig. 3a-d)

2.2.1 Taxonomy

Peters based his description of *B. mossambicus* on specimens from Mozambique Island and Sena (665 km apart). His Sena material has apparently been lost (Poynton & Broadley, 1985). Broadley (1966, 1971), Pienaar et al. (1976), and Poynton (1980) placed *B. adspersus* in the synonymy of *B. mossambicus* as a subspecies *B. mossambicus adspersus*. More recently, these two taxa have been treated as full species and intermediates have been interpreted as hybrids (Poynton, 1982; Poynton & Broadley, 1985; Lambiris, 1989a). Poynton and Broadley (1985) synonymised *B. mitchelli* Hoffman 1944, from Malawi, with *B. mossambicus* and suggested that the Angolan material previously referred to *B. mossambicus* by Parker (1934) and Poynton (1964), represents an undescribed species.

2.2.2 Distribution

Power (1926), who considered *B. adspersus* to be a synonym of *B. parvus* Hewitt 1925, recorded the distribution of *B. mossambicus* as "Mozambique, Zimbabwe, Botswana, Namibia, the Transvaal, Natal, and the Cape Province as far south as Grahamstown". Parker (1934), who recognised *B. adspersus*, gave the distribution of *B. mossambicus* as extending from "Zululand northwards through Mozambique , Zimbabwe and Angola to Malawi, Zaire and Tanzania". More recently, Poynton (1964, 1982), Poynton & Broadley (1985) and Lambiris (1989a&b) have recorded "pure" *B. mossambicus* from southern Tanzania, northern Zambia, southern Zaire (Shaba), Malawi and northern Mozambique, and have suggested that south of these areas, i.e. in southern Mozambique, Zimbabwe, southern Zambia, eastern Botswana, eastern Transvaal, and northern KwaZulu/Natal (from Empangeni, northwards), *B. mossambicus* hybridizes extensively with *B. adspersus*.

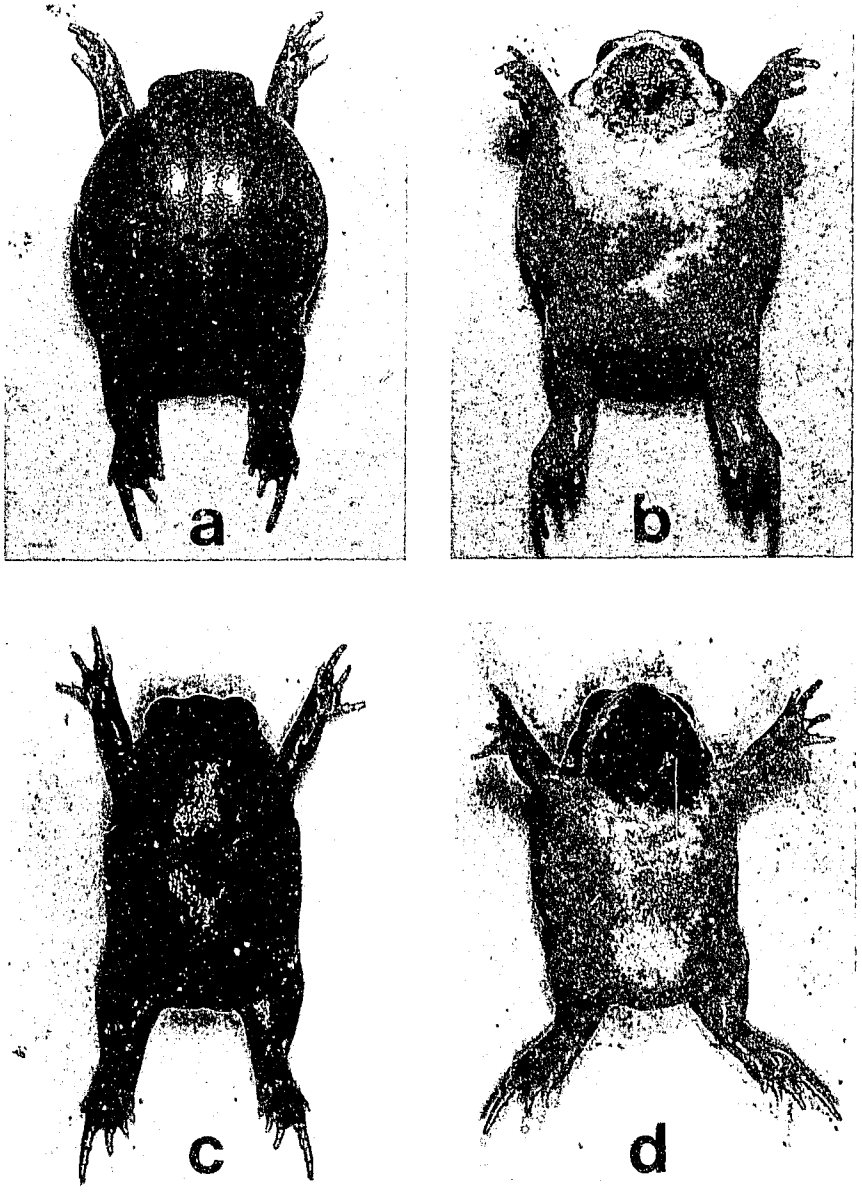

FIGURE 3. (a) *B. mossambicus* male from Loc.36 (Mozambique Island), dorsal; (b) ditto, ventral; (c) *B. mossambicus* male from Loc.40 (Nampula), Mozambique, dorsal; (d) ditto, ventral.

2.3 *Breviceps verrucosus* Rapp 1842 (Fig. 4 a-d)

2.3.1 Taxonomy and distribution

This species was relegated to the synonymy of *B. gibbosus* (L.) by Boulenger (1910) and Parker (1934) because of its granular skin, a character it shares with this species, but was resurrected by FitzSimons (1947) on the strength of additional material from Natal. Poynton (1964) synonymized *B. tympanifer* Hewitt with *verrucosus* by erecting a subspecies, *B. verrucosus tympanifer* Hewitt. He also regarded *B. rugosus* Power and *B. striatus* Hoffman as synonyms of the nominate subspecies *B. verrucosus verrucosus* Rapp.

Poynton (1964) restricted the distribution of *B.v. tympanifer* to the Eastern Cape south of the Kei River, while *B.v. verrucosus* was said to occur to the north of this, along the "lower eastern plateau slopes from the Transkei to Sabie in the eastern Transvaal". Lambiris (1989a:64) noted that the two subspecies are sympatric at Hillcrest, KwaZulu/Natal, and suggested that they might represent two separate species. However, he decided to avoid a taxonomic change "for the sake of nomenclatural stability". The question of the status of *verrucosus* and *tympanifer* is therefore unresolved.

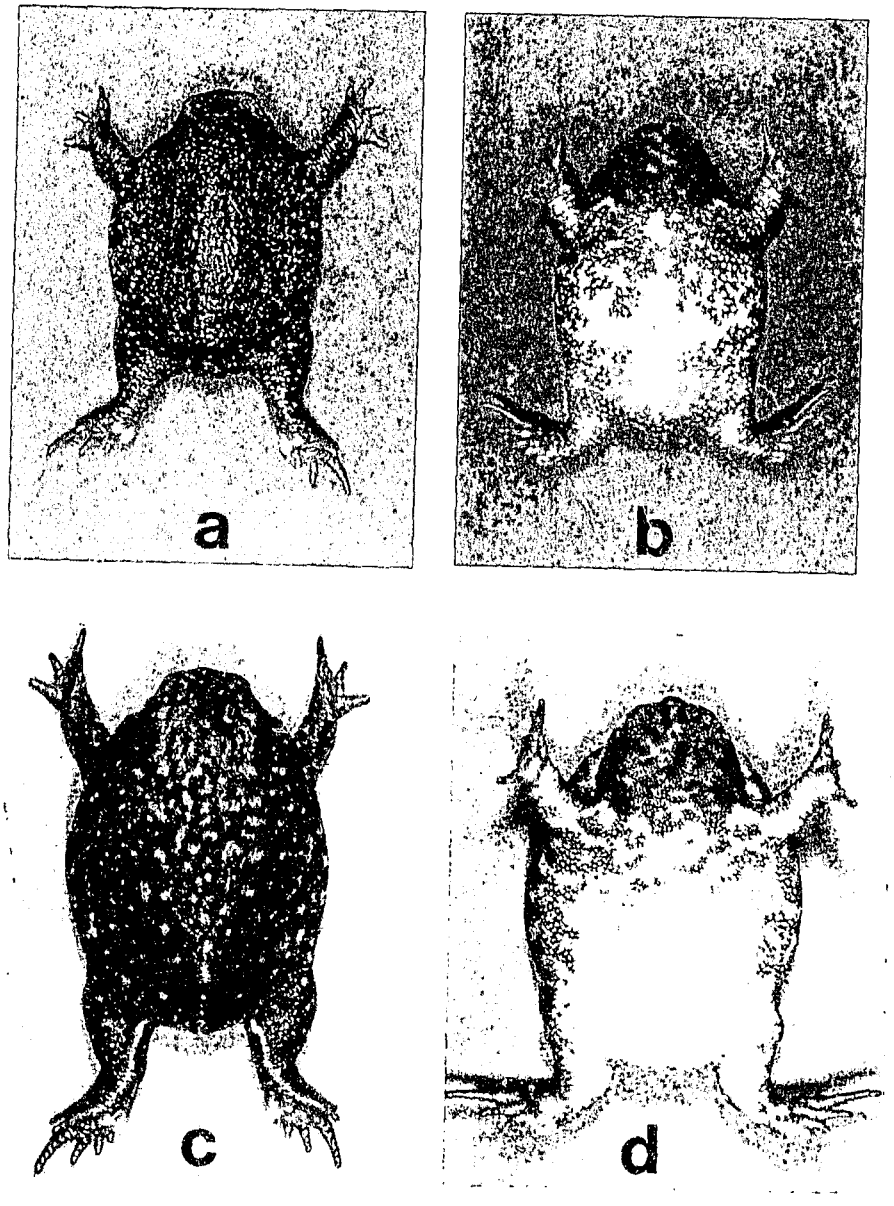

FIGURE 4. (a) *B.v. verrucosus* male from Loc.8 (Graskop), dorsal; (b) ditto, ventral; (c) *B.v. tympanifer* male from Loc.54 (Stutterheim), dorsal; (d) ditto, ventral.

2.4 *Breviceps sylvestris* FitzSimons 1930 (Fig. 5 a&b)

2.4.1 Taxonomy and distribution.

This species was described from specimens collected at Woodbush (Pietersburg District) and Haenertsburg; a single specimen from the Soutpansberg (Louis Trichardt) was also referred to this species. *B. sylvestris* is morphologically distinct from the parapatric *B. adspersus*.

The Soutpansberg population was subsequently assigned subspecific status, *B. s. taeniatus* Poynton 1963, and is known from a few localities in the vicinity of Louis Trichardt. The nominate subspecies, *B. sylvestris sylvestris* FitzSimons is associated with forests, from Woodbush and Haenertsburg southwards through Magoebaskloof to Tzaneen. It is not known whether the two subspecies come into contact in the intervening area but this is unlikely, due to the scarcity of suitable habitat: the geographic range of this species therefore appears to be quite limited.

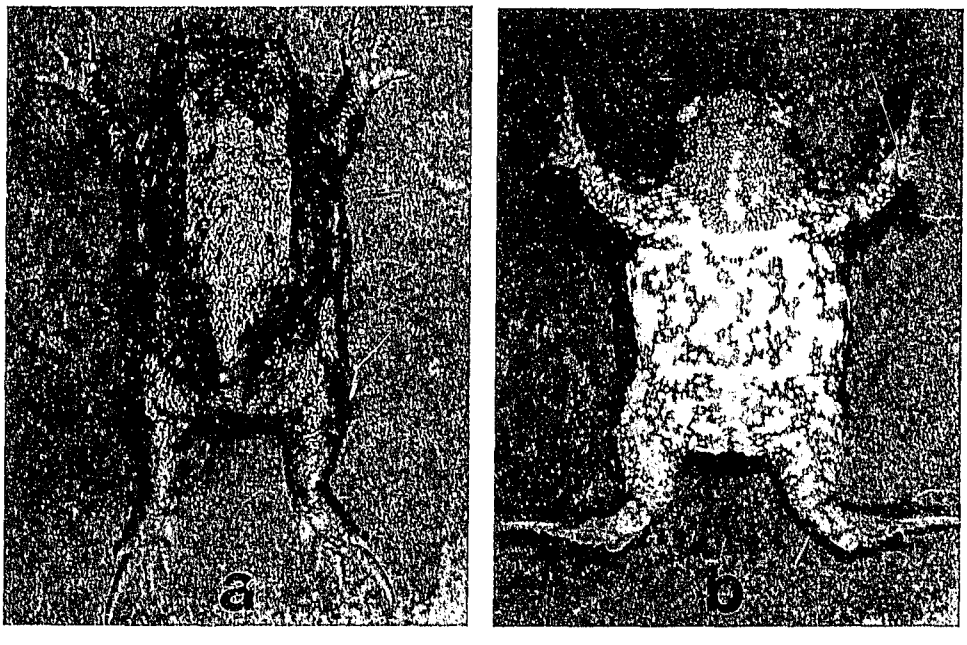

FIGURE 5. (a) *B.s. sylvestris* male from Loc.15 (Houtbosdorp 2), dorsal; (b) ditto, ventral.

2.5 *Breviceps poweri* Parker 1934 (Fig. 6 a&b)

2.5.1 Taxonomy and distribution

Parker (1934) described this species from Broken Hill (now Kabwe), Zambia. Poynton (1964) recognised *B. poweri* but cautioned that it might be conspecific with *B. adspersus*, while Poynton and Broadley (1985:527) stated that it was impossible, on morphological grounds, to "make a clean separation between" *adspersus* and *poweri* in specimens from Botswana and western Zimbabwe. They could find no evidence of hybridization between *B. poweri* and *B. mossambicus* in Malawi. *B. poweri* is recorded from Zambia, Malawi, south-eastern Zaire and northwestern Mozambique.

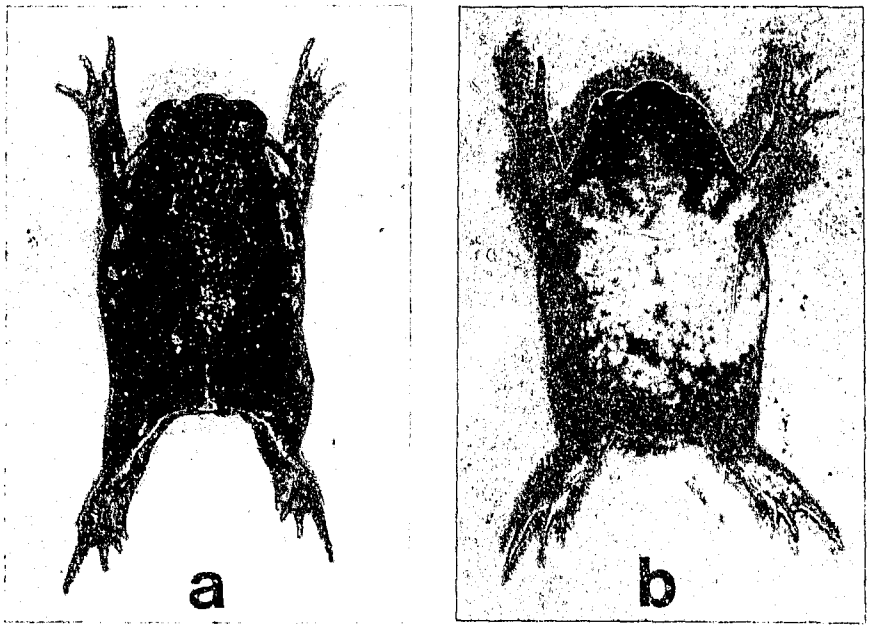

FIGURE 6. (a) *B. poweri* male from Loc.40 (Nampula), Mozambique, dorsal; (b) ditto, ventral.

3. Species concepts

3.1 Introduction

Several species concepts influence the practice of taxonomy today, and the interpretation of the data presented in this study will depend upon which of these concepts is followed. The species of *Breviceps* as currently defined are structural species, sensu Paterson (1993a:213), ie. they are "distinguished from others by a unique set of structural characters". Biochemical, metrical or cytological characters, identified by methods such as protein electrophoresis, restriction enzyme analysis of DNA or multivariate morphometric analysis are also structural characters and do not necessarily delimit genetical species. In the latter, "The crucial organisation within a species individual has to do with sexual reproduction", therefore, "the characters that are most directly symptomatic of species and speciation must be sought in the characters that relate to sexual reproduction" (Vrba 1985:x). This view will be expanded below in the comparison of two current genetical concepts: the Biological Species Concept (BSC) of the Dobzhansky/Mayr school, and Paterson's Recognition Concept (RC) which is applied in this study.

Species concepts may be grouped into three classes according to their underlying philosophy or theoretical basis, viz: typological, nominalist and genetical concepts (Mayr, 1963, 1969; McEvey, 1988).

3.2 The Typological species concept.

This predates evolutionary theory and is based on the Classical Greek philosophy known as Essentialism or Realism (Mayr, 1963, 1969; McEvey, 1988). Plato believed that natural phenomena such as species could be explained by the existence of perfect, immutable essences or ideas (eidos). The truth could be reached only by the precise definition of the eidos . Species definitions were based primarily on distinctive morphological traits, and variation was regarded as the result of imperfect manifestations of the eidos implicit in each species. Linnaeus was an essentialist, and his

hierarchical system of categories is a product of typological thinking. Sokal (1973) calls this the classical phenetic species concept, and it is also variously known as the morphological or taxonomic species concept.

The philosophical basis of this species concept has been superseded by evolutionary theory: however its influence still pervades taxonomy (Hull, 1965; Löve, 1964; Sokal, 1973) in the designation of certain specimens as "types", in the continued use of the Linnaean hierarchical system, and in the reliance, often entirely, on morphological characters in the description and diagnosis of species. The current taxonomy of *Breviceps*, with its emphasis on morphology, still reflects the influence of the typological species concept.

3.3 The Nominalist species concept.

According to this view individuals are real but species and higher taxa are merely abstractions created for convenience (Mayr 1970). Organisms are grouped on the basis of similarity, not affinity. Numerical taxonomists such as Sokal & Sneath (1963), who "make taxa" on the basis of the degree of observed similarity, and those who distinguish species and higher taxa on the basis of genetic distance, such as Avise (1976), Ayala (1975) & Lewontin (1974), are nominalists. Sokal (1973:361) regards the species of the numerical taxonomist as "only a quantification and refinement of the (classical) phenetic species of the orthodox taxonomist," differing only in its emphasis on populations and variation. This concept is incompatible with evolutionary theory and the genetical species concept, which are based on the premise that in nature, species are real entities, not abstractions.

3.4 Genetical species concepts.

During the Darwinian and post-Darwinian eras a new perspective developed in which life was seen as being dynamic and transitory. Evolutionary theory provided an alternative to creationist dogma as an explanation for the unity and diversity of life, and of the origin of species. The science of genetics which emerged from the work of Men-

del, de Vries, Bateson and Morgan was wedded to evolutionary biology by Fisher, Haldane and Wright in the form of mathematical population genetics (Hull, 1988).

The genetical concept of the species as a "gene pool" or "field of gene recombination" ie. a genetically integral natural unit within which evolutionary changes may occur, was articulated by early workers such as Wright (1932), Dobzhansky (1951) and Carson (1957). This new philosophy is based on genealogy, natural selection and change, and the flow of genes in populations. Species are now regarded as groups of genealogically related entities - thus, "instead of defining species, we discover them" (McEvey, 1988:16).

3.4.1 The Evolutionary Species Concept.

This broader genetical concept advocated by eg. Simpson (1961) and Wiley (1978), includes the time dimension in defining species boundaries and is therefore more relevant to palaeontology than to neontology. It also differs from the Biological Species Concept (BSC) in that it includes asexually reproducing organisms. Although this multidimensional concept approximates reality more closely than nondimensional concepts such as the BSC, the practical difficulty of determining the degree of genetic continuity (potential interbreeding) between allochronic and allopatric populations has led most biologists to favour the more restricted BSC (Mayr, 1963; Sokal, 1973; Vrba, 1985).

Nevertheless, the concept of the species as an "individual", in the logical sense: ie. unique and bounded in time and space (Ghiselin, 1966; Hull, 1976; Eldredge, 1985; Vrba, 1985), is useful: the concept of a species as a class implies that its members belong to the same species because they are similar, whereas the concept of the species as an individual implies that its members are similar because they belong to the same species. In other words, the elements that comprise the species "do so because of how they are organised and not because of any shared similarity" (Hull 1980:313). This perspective is also a feature of the Recognition Concept (3.4.4), which focuses attention on the features which are responsible for the maintenance of genetic coherence within the species.

3.4.2 The Phylogenetic or Genealogical Species Concept.

This concept is derived from the perspective of phylogenetic systematics (Hennig, 1966) and its modern counterpart, cladistics. It is tied to the general theory "that evolution occurs and produces a hierarchy of monophyletic groups" (Donoghue 1985:175). According to this concept, species should first be identified using a grouping criterion and then ranked according to a ranking criterion. The criterion for group membership is monophyly, and such groups are identified on the basis of shared derived (apomorphic) characters. As regards ranking, Donoghue (1985) recommends that every one of the smallest monophyletic units that can be identified be allocated species rank: these would probably correspond to the actually interbreeding evolutionary populations of the BSC. The designation of infraspecific taxa and categories, characteristic of the BSC, would therefore be unnecessary. McKitrick and Zink (1988) have proposed the implementation of this concept in ornithology.

While Donohugh (1985) is correct in pointing out that morphological similarity does not necessarily indicate monophyly, he errs in stating that the potential for interbreeding does not indicate monophyly. If speciation is accompanied by changes in the characters of the Specific-Mate Recognition System (SMRS), then such characters are undoubtedly derived (synapomorphic): therefore the presence of a common SMRS is evidence of a monophyletic group, regardless of the degree to which the populations may have diverged morphologically or ecologically (Masters and Spencer, 1989). Cladistical analysis is obviously subjective as its result depends upon which characters are used. Also, the strict application of this concept would probably result in a plethora of species. As Donohugh (1985) points out, the grouping criterion is relatively easy to apply, but ranking is more difficult and it would probably be impossible to achieve equivalence between groups in this regard. Hybridization also poses theoretical and practical difficulties in the application of the phylogenetic concept.

3.4.3 The Biological Species Concept (BSC).

In the course of its prolonged advocacy by prominent evolutionists such as Dobzhansky (1935, 1937, 1951, 1970, 1976), and Mayr (1942, 1949, 1963, 1970, 1976) this genetical concept has achieved wide acceptance among biologists and constitutes

the current paradigm. Mayr (1969:26) defined biological species as "**groups of interbreeding natural populations that are reproductively isolated from other such groups**". The most important components of this concept are thus genetic cohesion, populations, and reproductive isolation. Other significant consequences of this definition are: that the BSC is restricted to sexual organisms, and that it is a relational concept, in that species are perceived as being reproductively isolated from other species (Mayr 1963, Paterson 1985, Masters & Spencer 1989). Mayr (1963:17) calls the BSC a "nondimensional" concept as it is "based on the relation of two coexisting natural populations... at a single locality and at the same time (sympatric and synchronous)".

Proponents of this concept have assigned a key role to so-called reproductive isolating mechanisms in maintaining the genetic integrity of the species (eg. Dobzansky, 1970; Mayr, 1963, 1969; Littlejohn, 1981), with the result that this function has come to be regarded by many as implicit in the concept. Paterson (1980:330) highlighted this aspect of the BSC by dubbing it the "Isolation Concept."

3.4.4 The Recognition Concept (RC).

This genetical concept of species has been propounded and defended in a series of papers by Paterson (1973, 1978, 1980, 1982a, 1985, 1993a&b), Lambert & Paterson (1984) & Macnamara & Paterson (1984). Paterson (1985:25) defines a species as "**that most inclusive population of organisms that share a common fertilization system**". Like the BSC, this concept also emphasises genetic cohesion and population structure, and is restricted to sexual organisms. Paterson (1978, 1980, 1985) recognizes within the fertilization system of motile, sexual organisms, a sub-set of signal-response characters which he calls the **Specific-Mate Recognition System (SMRS)**. This comprises adaptations which "ensure effective syngamy within a population of organisms occupying their preferred habitat. The characters of the SMRS are adapted to function efficiently in this preferred habitat" (Paterson 1982a:54).

3.4.5 Fundamental differences between the RC and the BSC.

Although the RC has won some support (eg. Vrba, 1985; Templeton, 1987; Lambert & Henderson, 1986; Masters et al., 1987; Hulley et al., 1988; Walter, 1988; Masters & Spencer, 1989), it has also met with resistance, particularly from those who support the concept of speciation by reinforcement (eg. Templeton, 1987; Coyne et al., 1988). Others (eg. Raubenheimer and Crowe, 1987) feel that the RC is not sufficiently different from the BSC to justify its recognition as an alternative concept. Lambert and Paterson (1984), Macnamara & Paterson (1984), Masters et al. (1987), and Masters and Spencer (1989) have described the fundamental differences between the two concepts and have discussed their implications and consequences.

Two important differences between the BSC and RC are:

a) The BSC reflects a teleological viewpoint.

In Dobzhansky's (1972:665) view, "pre-mating isolating mechanisms are mostly not accidental by-products of genetic divergence but adaptive contrivances that guard against the breakdown of functionally coherent adaptive systems", ie. ad hoc, adaptive devices. Similarly, Mayr (1963:89) regards isolating mechanisms as "a whole set of special devices by which the gap between species is maintained", ie. "They are *ad hoc* mechanisms" (Mayr 1963:548). As late as 1976, Dobzhansky still maintained that "species are not accidents but adaptive devices through which the living world has deployed itself to master a progressively greater range of environments and ways of living".

Paterson (1982b:274) and Masters et al. (1987:536) have pointed out that Dobzhansky's view of the origin and function of isolating mechanisms reflects "a teleological and idealistic philosophical view of species", as well as a disposition towards group selection theory (see also Ghiselin, 1969; Hull, 1965, 1988; Monod, 1970; Skolimowski, 1974; and Williams, 1966 for comments on the persistence of teleology in natural science).

More recently, Mayr (1991:2-3) has asserted that in the new evolutionary synthesis, deterministic thinking of this kind has been "replaced by a recognition of s ·· aastic processes and of pluralism. Such evolution is not necessarily progressive; it does not strive toward perfection or toward any other goal; it is opportunistic, hence unpredictable". Referring to the teleological concept of natural selection as a process driving towards adaptation, he states (1991:4) "I am sure we all reject such a teleological explanation". Other defendants of the BSC such as Coyne et al. (1988) also assert that most modern evolutionists do not assume direct selection for isolating barriers.

However, many workers still continue to search for evidence of reproductive character displacement to support the model of speciation by reinforcement (eg: Ayala et al., 1974; Bush, 74, 1975; White, 1978; Littlejohn, 1981; Templeton, 1987 and Donoghue, 1987). Thus the view that isolating mechanisms evolve in order to "protect the genetic integrity" of the species, still finds support among proponents of the BSC.

In contrast, the RC lacks a teleological bias since it calls for nothing more than individual selection. Paterson (1986:63) argues that species are not 'adaptive devices', ie. products of selection for diversity, "but are incidental consequences of adaptive evolution"."The Recognition Concept does no' assign a major driving role to interspecific competition. Consequently, reproductive character displacement is considered unlikely" (Paterson, 1986:64). Isolating mechanisms are rather seen as "an incidental *effect* resulting from the adaptation of the characters of the fertilization system, among others, to a new habitat or way-of-life" (Paterson, 1985:26). This view is supported by Moore (1957), Williams (1966), Ehrlich & Raven (1969), Sokal (1973), Vrba (1985), Carson (1987), Templeton (1987), Butlin (1987) and others.

Chandler & Gromko (1989:120) have argued that the BSC is acceptable if a logical distinction between concept and process is maintained ie. the term reproductive isolation is acceptable as long as it is not used in the context of the speciation process. They point out that "conceiving of species as reproductively isolated units does not demand that isolation be adaptive or arise through a process of reinforcement". Nevertheless,

the word *isolate* is more likely to be interpreted as a verb than a noun and the action of isolating implies the presence of a process or mechanism, hence "isolating mechanisms". Thus the BSC subtly encourages an association between the concept of species and the process of speciation. Donoghue (1985) also draws attention to the confusion and controversy that has resulted from linking the BSC to a theory of causation.

Chandler and Gromko further suggest that the term "isolating mechanism" be replaced by "isolating effect" or "isolating barrier". A similar change was implemented by Littlejohn (1981:328) who replaced the term "reproductive isolating mechanism" with "homogamic mechanism" in order to place emphasis on the individual, because he felt that "the concept of reproductive isolation, which is based on the maintenance of the genetic integrity of supraindividual units of biological organization.... is no longer accepted as operational". However he continued to associate these homogamic mechanisms with "the maintenance of genetic discontinuity in coexistent biparental systems", and thus attributed the same supraindividual function to them as is implied by the term "reproductive isolating mechanism". Homogamic mechanisms are therefore "nothing but isolating mechanisms in disguise" (Masters et. al., 1987:535). Changing the terminology does not necessarily change the underlying teleological dogma of the BSC, which represents a subjective interpretation of nature.

The RC is free of the inconsistencies and contradictions that beset the BSC. In terms of the RC, the characters which delimit the gene pool are restricted to the adaptations of the SMRS, which serve the express and sole purpose of ensuring successful syngamy: they have not evolved in order to ensure reproductive isolation.

b) The BSC is a relational concept.

Another problematical aspect of the BSC is that it defines species relationally, ie. in terms of their reproductive isolation from other species (Mayr, 1963; Paterson, 1981, 1982a, 1982b, 1985, 1986; Macnamara & Paterson, 1984; Masters et al., 1987; McEvey, 1988; Masters & Spencer, 1989). This makes it difficult to explain how isolating mechanisms arise since, according to the only generally accepted model, spe-

ciation takes place in small **allopatric** populations. While Dobzhansky believed that isolating mechanisms could arise in sympatry in response to the deleterious effects of hybridization, Mayr (1963:548) suggested that they arise as incidental by-products of allopatric divergence but are further modified in sympatry to better serve their isolating function: "The thesis of the origin of reproductive isolation as a by-product of the total genetic reconstitution of the speciating population is consistent with all the known facts. Nevertheless natural selection does play a role in the improvement of some of the isolating mechanisms, only it concerns subsidiary isolating mechanisms. The primary, basic one must be fully efficient when contact is first established." The "improvement" of existing isolating mechanisms is only feasible in situations where there is extensive overlap between two populations and Mayr's model fails to explain how such modified characters could spread and become fixed in populations which overlap only partially (Moore, 1957; Paterson, 1978, 1982a). Scoble (1985:33) notes that in the absence of a method based on a fundamental explanation, whereby potential interbreeding can be inferred, "only *actually* interbreeding populations can be regarded as conspecific" The BSC, as a nondimensional concept, is thus of no value to the taxonomist, who needs to assess the status of allopatric populations, ie. a multidimensional concept.

The RC is not a relational concept. Therefore "a species defined in terms of positive recognition always has an objective existence, irrespective of the existence of other such populations" (Masters et al., 1987:535). Coyne et al. (1988), and Templeton (1987) dispute this but Masters and Spencer (1989) effectively refute their arguments. They note (p273) that "the importance of a non-relational species concept lies in the way it focuses attention on those features of a species which are crucial to its continued existence and evolution.", ie. the SMRS.

3.4.6 Important implications of the RC.

The implications of the RC with regard to phenomena such as geographical variation, hybridization and polyploidy, may be more clearly understood if one considers the origin of the SMRS, ie. the process of speciation. Paterson (1985:26) envisages the process as follows: "While members of a species are occupying their normal habitat

the characters of the fertilization system, as well as other adaptive characters, are all maintained under stabilizing selection". This buffering of adaptive characters can be most easily overcome if a small population becomes restricted to a new habitat. "Any adaptive characters, including those of the SMRS, which are now less well adapted will become subject to directional selection". Once they have become "appropriate and effective under the new conditions, they will return once more to the control of stabilizing selection". Speciation will have occurred "if the new fertilization system has become sufficiently different from that of the members of the parent population, for then the new fertilization system will delimit a new field for gene recombination".

Paterson notes, however, that the characters of the SMRS are more resistant to change than other adaptive characters "because the co-adaptation that exists between the mating partners with respect to signals and receivers constitutes an effective buffer to change". Henderson and Lambert's (1982) and Lambert and Henderson's (1986) studies of the stability of the SMRS of *Drosophila melanogaster* sampled from populations around the world, support this view. This stability of the SMRS is likely to be more pronounced in vagile generalist species than in non-vagile species with more specialised habitat requirements.

In summary, Paterson (1982a:54) states: "Hence, it follows that a new species will have arisen when all members of a small, isolated, sub-population of a parental species have acquired a new SMRS, which facilitates the achievement of syngamy under the new conditions, and which, quite fortuitously, makes effective signalling impossible between members of the daughter and parental populations".

In accordance with this perspective the following **implications** and **predictions** of the RC may be identified:

a) "Under the recognition concept, provided one succeeds in identifying the characters of the SMRS, one can test the hypothesis that allopatric populations belong to one species", whereas this is not possible in the relational and non-dimensional BSC (Vrba 1985:xi).

b) Interpopulation variation in the SMRS can occur, but speciation is restricted to small, allopatric populations. Speciation in sympatry is not supported.

c) Speciation and the acquisition of a new niche go hand in hand: the normal or preferred habitat of a species is of fundamental importance in the RC, and is likely to be very similar to the habitat in which the species arose. A similar process was envisaged by Moore (1957:337): "the most important aspect of this process (speciation) is genetic divergence associated with adaptation to the local environment." Because of the adaptation of the SMRS to the preferred habitat, the RC also predicts that a species will respond to changes in climate by following (tracking) the moving environmental contours and so its distribution will expand and contract accordingly, ie. "organisms would be more likely to move to better areas of the preferred habitat than remain immobile as their environment changes, unless they were prevented from doing so" (Paterson, 1982a:56). It is only when the population size falls below a critical level, e.g. in a small isolated population, that changes are likely to take place in the SMRS and the habitat preferences of a species (Masters et al., 1987).

d) Speciation is preceded and followed by stasis (sensu Eldredge and Gould, 1972), and therefore the RC fits the model of punctuated equilibria. This is because the fertilization system is very stable, due to the co-adaptation of the male and female components, and its adaptation to the normal habitat of the species.

e) Hybridization is seen from a different perspective. Two allopatric populations which, on becoming sympatric, produce infertile hybrids are considered to be conspecific under the RC since they still share a common fertilization system, but separate species under the BSC since, as Templeton (1987) and Butlin (1987) have pointed out, the two populations are genetically separate and cannot be regarded as "a field for gene recombination". Since the gene-pool concept is regarded as "the heart of any genetical species concept" (Paterson, 1985:21), the above example reveals an apparent flaw in the RC. Masters and Spencer (1989) argue that such situations are impermanent because the post-mating mechanisms would be rapidly eliminated by selection pressure acting against the inferior hybrids. Consequently, the two species (as recog-

nised under the BSC) would revert to one species. Permanence and irreversibility are necessary characteristics of species in terms of genetical and evolutionary theory. In this respect the RC is superior to the BSC in its interpretation of hybridizing populations.

In less extreme cases, where hybrids have reduced viability but are not totally infertile, genes are still able to move from one population to the other, ie. the populations still share a common gene pool. Again, natural selection would act against any genes causing hybrid disadvantage and the two populations would become more homogeneous. Advocates of both the RC and the BSC would regard such populations as belonging to a single species.

f) Genetic discontinuity due to the phenomenon of polyploidy is treated, under the RC, in the same way as other post-mating isolating mechanisms. Paterson (1985:26-27), using an example of an autotetraploid, argues that its gene pool is merely a subset of the gene pool of the diploids and in terms of population genetic theory this is an example of heterozygote disadvantage. Because they still share a common SMRS, the tetraploids would be rapidly eliminated. According to this theory, one would expect a tetraploid which is sympatric with its diploid ancestor to have a different SMRS.

g) Coyne et al. (1988) argue that whereas the RC predicts stability in SMRS characters throughout the range of a species, intraspecific variability is a common phenomenon, even in SMRS characters. Masters and Spencer (1989) show that this is an overstatement of the RC prediction and query the validity of their examples of geographic variation in SMRS characters. They note that in any description of variation in the SMRS, the specific features involved in recognition must be identified and irrelevant characters eliminated. Paterson (1993a) points out that many of the examples used to illustrate variation in the SMRS are morphological species: therefore the observed variation may be due to the presence of more than one genetical species. He also notes (p215) that adherents of the RC do not postulate invariance, but rather the operation of stabilizing selection on the variation that may be present, and adds, "The low variability of which I spoke relates to those individuals that transmit genes to the

next generation: the population that has already traversed stabilizing selection", i.e. he excludes aberrant individuals (outliers) which, he argues, will be eliminated by natural selection.

3.4.7 The applicability of the RC to taxonomy.

The practice of taxonomy involves assigning taxa (populations or groups of populations) to categories such as species or subspecies. The species category is defined by the species concept (Mayr, 1963).

Several authors have pointed out that the classical phenetic (typological) species concept is still applied in the orthodox taxonomic treatment of most groups of organisms today due to the absence of information needed for the application of genetical concepts (Sokal, 1973; Donohugh, 1985). On the other hand, Mayr (1969:25) argues that "there is a complete difference between basing one's species concept on morphology and using morphological evidence as inference for the application of a biological species concept". Löve (1964) concurs: "certain morphological character combinations may often be relatively safe indicators of reproductive principle". However Scoble (1985:33) points out that potential interbreeding is best assessed by an examination of the SMRS. "This is not a direct genetic explanation, but it is a great deal better than inferring potential interbreeding from such sources as general morphological similarity."

The fact that SMRS characters have not been identified in the vast majority of sexually reproducing organisms begs the question: "What would Paterson have systematists do with morphologically discrete populations in the absence of critical information on the SMRS" (Raubenheimer and Crowe, 1987:532)? Paterson (1993a:224) feels that "a universal concept of species is needed within taxonomy" and that "genetical concepts are not appropriate in the field of taxonomy largely because they do not apply to uniparental organisms". McEvey (1988:11) also contends that taxonomy should be "clearly distinguished from evolutionary and population genetical studies" ie. the RC is relevant to evolution and population genetics, but not necessarily to taxonomy. It is unlikely that the universal concept envisaged by Paterson would provide

a standard that reflects reality.On the other hand, Masters and Spencer (1989) "feel that the consequences of sex in biparental species are so profound as to warrant the emphasis of sexual reproduction among these organisms, and for this reason, a genetic species concept should be retained for them".

It is clear that genetical species are real entities in nature, whereas typological and nominalist species are nothing more than artificial conveniences. Mayr (1969:24) regards the latter as "species that are biologically, and hence scientifically, meaningless". Thus an accurate picture of biological diversity can only be obtained through the application of a genetical species concept. Surely our improved understanding of the origin and manifestation of biological diversity, gleaned from the fields of population genetics and evolution, should be applied in taxonomy to produce a more natural classification? The popularity of the BSC certainly indicates that the majority of biologists agree with this opinion. The assigning of taxa to species on the basis of morphology must therefore be seen as a practical temporary measure resorted to in the absence of adequate information, and where possible, taxonomists should use diagnostic characters which enable them to identify the real entities in nature: genetical species.

The RC presents the most parsimonious and logical view of genetical species, and is consistent with the known facts. It defines the limits of the field for gene recombination in a positive fashion and yields a clearer vision of evolutionary "function" versus "effect", whereas the BSC is actively misleading (Templeton, 1987).

Therefore, in this study,an attempt will be made to delineate genetical species as defined by the Recognition Concept, by using the advertisement call as a diagnostic character. The status of existing taxonomic species will be discussed in the light of the results.

4. Advertisement calls

4.1 Vocal communication in anurans.

Sound provides the most effective channel for long distance communication in frogs, but in some species other sensory modalities such as substrate borne vibrations (Lewis & Narins, 1985), visual (Wells, 1978), tactile (Wells, 1977b) and chemical (Straughan, 1967) cues may be used over moderate and short distances. Reviews of this field of study (eg. Blair, 1958,1963; Bogert, 1960; Duellman & Trueb, 1985; Gerhardt, 1988; Littlejohn, 1977; Rand, 1988; Straughan, 1973; Wells, 1977a & b, 1988) document its development from an early descriptive and theoretical phase, to an experimental phase involving behavioral and neuro-physiological studies of call discrimination.

Various aspects of vocal communication have been studied, viz: the functional morphology of the vocal apparatus, the neurophysiology of the auditory apparatus, the physical structure of the signals themselves, and the information that is being communicated. Frog calls may therefore be classified in different ways, depending upon which aspect of the call is being studied (Rand, 1988).

A classification of calls in terms of **function and situation**, was proposed and modified by Bogert (1960), Littlejohn (1977), Wells (1977a) and Rand (1988). The last author distinguishes between **advertisement, courtship, aggressive, release** and **distress** calls (pp 419-20). Territorial calls (sensu Bogert, 1960; Littlejohn, 1977) and encounter calls (sensu McDiarmid and Adler, 1974) are signals emitted during higher intensity calling or short range male-male interactions, respectively, and Rand (1988) classifies both as aggressive calls. In considering function it is useful to distinguish between the message and the meaning of a frog's call. The message (information) contained within the signal may include species identity (recognition component), sex, size and location, whereas its meaning is contingent upon the identity of the recipient (Rand, 1988).

The use of the term "**advertisement call**", which refers to long-range acoustic signals directed at male and/or female recipients, was advocated by Wells (1977a&b) and is now widely used. The advertisement call is usually produced by a breeding male and functions to attract a conspecific female and/or announce his presence and position to surrounding conspecific males.

4.2 Advertisement call structure.

In design, advertisement calls (or "notes" sensu Duellman and Trueb, 1985:89) are discrete, and ritualised (Rand, 1988). They usually contain an element of redundancy, ie. calls may be repeated a number of times in a bout of calling. Within a call bout, the calls may be evenly spaced or grouped. The rate at which calls are produced (call repetition rate) may be constant throughout the call bout or may change during the bout. Call repetition rate may also be affected by factors such as body temperature, the proximity of calling males, or the approach of a female.

Each call may be unpulsed or pulsed: ie. its waveform may be uniform throughout or it may be amplitude modulated to form a temporal series of sound pulses (eg. a trill or buzz, sensu Wells, 1977b). Call structure appears to be adapted to certain environments: eg. pulsed calls are more suited to open situations, such as grasslands, than are tonal signals (Morton, 1975; Littlejohn, 1977; Wiley & Richards, 1978; Passmore, 1981), while the short, unpulsed whistle of *Heleophryne purcelli* occupies a narrow frequency range and propagates more effectively against a background noise of running water, than that of *H. regis* which breeds in a quieter environment and has a longer call with the sound energy spread over a wider frequency range (Passmore, 1985).

In some species the call is undifferentiated (monophasic), but may elicit different responses, depending upon the context or the sex of the recipient. In other species, calls are biphasic: ie. the call is divided into two temporally separate and structurally dissimilar parts. In such cases, information directed at females may be temporally separated from that directed at males (eg. *Eleutherodactylus coqui*, Narins & Capranica, 1976; Stewart & Bishop, 1994; *Physalaemus pustulosus*, Rand & Ryan, 1981; Ryan,

1983a; and *Afrixalus brachycnemis*, Backwell, 1988).

In analysing the structure of the call it is convenient to distinguish between temporal characters and spectral characters, although these two groups are interdependent: This is clearly seen in call discrimination experiments involving synthetic calls, where "changes in temporal properties inevitably lead to changes in spectral properties" (Gerhardt, 1988:473). Another example is seen in rapidly pulsed calls, where sidebands are generated on either side of the dominant frequency band, and the spacing between the bands is directly proportional to the pulse rate (Camhi, 1984; Gerhardt, 1978a, 1988).

4.3 The effect of temperature and body size on advertisement call structure.

4.3.1 Temperature

Temperature has a significant effect on a number of call parameters. This has to be taken into account when comparing calls that were recorded at different temperatures. If the data base is large enough it is possible to calculate a correction factor from a least squares linear regression analysis (ie. a temperature regression coefficient) which allows one to adjust the data to a common temperature (eg. Blair, 1955; Littlejohn, 1965; Forester, 1973; Fouquette, 1975; Ralin, 1968; Gayou, 1984). In general, variation in temperature has a greater effect on temporal call properties than it does on spectral properties.

Call characters which are **positively** correlated with temperature are **call repetition rate** (Blair, 1955, 1958), **pulse rate** (Littlejohn, 1965, 1968; Zweifel, 1968; Schneider, 1974; Gerhardt, 1978b; Sullivan, 1982; Gayou, 1984) and, to a lesser extent, **dominant frequency** (Blair, 1955, 1958; Blair & Littlejohn, 1959; Passmore & Telford, 1983; Gayou, 1984).

Call characters which are **negatively** correlated with temperature are **call duration** (Blair, 1958; Littlejohn, 1968; Schneider, 1974; Gayou, 1984; Littlejohn & Harrison, 1985), ***pulse duration*** (Gayou, 1984), ***intercall interval*** (Schneider, 1974; Gayou,

1984), and to a lesser extent, **pulse number** (Schneider, 1974; Gayou, 1984).

The auditory sensitivity of female frogs is also temperature dependent (Moffat & Capranica, 1976; Gerhardt, 1978b, 1982; Hubl & Schneider, 1979; Mohneke & Schneider, 1979; Gerhardt & Mundry, 1980). Gerhardt (1978b, 1982) and Gerhardt & Doherty (1988) demonstrated the presence of temperature coupling in *Hyla versicolor*: females preferred the calls of males whose body temperature was similar to their own. Thus variation in temperature affects the emitter and the receiver systems in a qualitatively similar way. Gerhardt & Doherty (1988) found that in *H. versicolor*, temperature coupling in respect of pulse rate was not uniformly developed over a wide range of temperatures, in that females at low temperatures were more selective than warm females. This may be explained by differences in the response of the two papillae of the inner ear to temperature (see 4.5.3 below).

In the context of the Recognition Concept of species, temperature coupling may be viewed as a refinement of the mate recognition system, which increases its efficiency, rather than a mechanism which has evolved to prevent mismating, as was suggested by Gerhardt (1978b).

4.3.2 Body size

The variation in body size within a population has an influence on both spectral and temporal call characters as well as on the intensity of the call. Call characters which are **positively** correlated with body size are **call duration** (Zweifel, 1968; Fairchild, 1981) and **call repetition rate** (Forester & Czarnowsky, 1985).

Characters which are **negatively** correlated with body size are **dominant frequency** (Zweifel, 1968; Ryan, 1980, 1983a; Arak, 1983a; Passmore & Telford, 1983; Doherty & Gerhardt, 1984; Robertson, 1986; Gerhardt et al., 1987), and **pulse rate** (Fairchild, 1981). The peak **intensity of the call** was found to be lower in small males than in large males in a number of species (Loftus-Hills & Littlejohn, 1971b; Gerhardt, 1975; Arak, 1983b). It has been suggested that the correlation between size and various call parameters enables females and other males to assess the caller's potential as a mate or

adversary respectively (Fairchild, 1981; Ryan, 1980, 1983a; Arak, 1983b; Ramer et al., 1983).

4.4 The mate recognition function of the advertisement call.

It has long been recognised that frog advertisement calls are species specific, and they have been widely used as diagnostic taxonomic characters (Blair, 1958, 1964; Littlejohn, 1968, 1969; Schiøtz, 1973; Passmore, 1981, 1985).

When we state that a call is "species specific", we also draw attention to its structure **in relation to** other species. This relational viewpoint has been the *raison d'être* behind numerous advertisement call studies aimed at substantiating concepts such as reproductive isolation (involving isolating mechanisms) and speciation by reinforcement (Blair, 1955, 1958; Littlejohn, 1959, 1965, 1976; Loftus-Hills and Littlejohn, 1992) and its influence can be seen in many other papers dealing with frog advertisement calls (eg. Konishi, 1970; Gerhardt, 1982, 1988; Walkowiak, 1988). The observation that species which use the same breeding habitat usually have markedly different advertisement calls (Littlejohn & Martin, 1969; Konishi, 1970; Straughan, 1973) has often been used in support of these concepts. In reassessing some of these earlier studies, Loftus-Hills, (1975); Nevo & Capranica (1985), Ryan & Wilczynski (1991) and Paterson (1993a) have concluded that they contain little, if any, evidence to support the reproductive character displacement hypothesis: this relational viewpoint is, in any case, incompatible with the Recognition Concept of species. The term "mate recognition" is therefore more appropriate than "species identity" if the primary function of the advertisement call is to bring individuals of the same species together to mate.

Two-choice discrimination experiments were first used by Littlejohn & Michaud (1959) to confirm the mate recognition function of the advertisement call. A frog which responds differentially to one of two signals must be able to perceive a difference, and in this sense it is making a choice. Female frogs were presented with the choice of a conspecific and a heterospecific advertisement call, and responded differ-

entially to the former.

Initially, these experiments were conducted using natural calls (Littlejohn, 1960; Littlejohn et al., 1960; Awbrey, 1965; Littlejohn & Loftus-Hills, 1968; Littlejohn & Martin, 1969; Straughan, 1966, 1975), but in later studies workers used synthetic calls and were able to vary individual spectral and temporal call components in order to determine their relative importance in recognition (see 1.4.5 below). It was found, under experimental conditions, that the females were capable of a fine degree of discrimination, preferring the calls of their own population (local "dialects") to those of other populations, even though the calls differed only slightly (Capranica et al., 1973; Nevo & Capranica, 1985; Ryan et al., 1990; Ryan & Wilczynski, 1991).

But the discrimination experiments also showed that in the absence of a choice they would respond to heterospecific calls or to artificial stimuli differing significantly from their conspecific advertisement calls (Littlejohn et al., 1960; Oldham & Gerhardt, 1975; Gerhardt, 1982, 1988). Furthermore, Backwell and Jennions (1993) found that when females of *Hyla ebraccata* were presented with a choice between the calls of *H. phlebodes* and *H. microcephala*, they showed a significant preference for the former. When no alternative was offered, they also responded to the call of *H. phlebodes*.

These observations show that although females appear to "recognise" conspecific calls when presented with a choice, the conspecific call does not elicit an all-or-none response. Instead, the response appears to be graded, ie. a female may perceive and respond to a variety of signals, and "It is only the relative attractiveness of conspecific calls that maintains the pattern of conspecific mating" (Backwell & Jennions, 1993:1249).

In a natural situation, however, it is unlikely that a receptive female would find herself in a chorus that lacked conspecific males. Also, while the advertisement call is clearly the fundamental constituent of the SMRS in most frog species, it does not function in isolation but is part of a broader fertilization system (Paterson, 1985) in which numer-

ous other factors, such as environmental cues, co-ordination of the reproductive cycles of conspecific males and females, the call site, and visual and tactile cues, play subsidiary roles in bringing conspecifics together.

The functional aspects of *Breviceps* advertisement calls were not investigated in this study because amplexing pairs adhere to one another by means of a sticky secretion: the forced separation of an amplexing pair is traumatic and sometimes damages the skin, causing bleeding. Furthermore, amplexing pairs are seldom found because they are terrestrial breeders and oviposition takes place below the surface. It is therefore difficult to obtain receptive females for phonotaxis experiments.

On two occasions female *B.a. adspersus* were seen to approach calling males. On becoming aware of the female's presence, the male increased his call rate and left the call site to intercept her. Although neither of these interactions culminated in amplexus, possibly because of the presence of the observer, these observations support the assumption that the primary function of the call in *Breviceps* is to attract a conspecific mate.

All other assumptions relating to the function of the call or call components in *Breviceps* must, of necessity, be based on current knowledge of other taxa reviewed below.

4.5 Call parameters implicated in mate recognition.

Once the mate recognition function of the advertisement call had been established, attempts were made to determine which of the various call parameters are involved in mate recognition. Littlejohn (1959) predicted that species co-existing in large breeding assemblages would differ in at least one of these parameters.

4.5.1 Sound pressure level (SPL).

Several workers have found that at moderate SPLs, female frogs respond selectively to a call 2-6 dB louder than an alternative (Fellers, 1979; Arak, 1983b; Gerhardt, 1987; Bishop et al., 1995). Arak (1983b) played calls differing in intensity to female

Bufo calamita, but broadcast the louder call at a greater distance from the subject, so that the call intensities of both calls were equal by the time they reached the female. He found that the females did not show a significant preference and concluded that the females could not assess absolute amplitude. It appears that in this species the female merely responds to the loudest perceived call which, in natural situations, is usually the nearest male.

By calling from elevated positions, frogs may reduce ground attenuation and the scattering of sound by vegetation, and counteract the effects of shadow zones caused by wind or temperature gradients (Wiley & Richards, 1978). This enables them to project their calls further than is possible at the surface.

Call intensity is an important factor to consider in the design and interpretation of call discrimination experiments because changes in intensity can sometimes cancel or even reverse a female's preferences in respect of other call variables (see below).

4.5.2 Temporal features.

With regard to **call repetition rate**, the females of most species tested show a preference for repetition rates above the mean (Gerhardt, 1988). Female *Hyla microcephala* prefer a call with a higher repetition rate over one with a lower rate but this preference is negated or reversed if the SPL of the former is lowered (Schwartz, 1986). Sullivan (1983) found that mating success was greater in males of *Bufo woodhousei* that called at a faster rate than in slower callers. Lopez & Narins (1991) found that females of *Eleutherodactylus coqui* showed a strong preference for a relatively high call rate.

It has been noted that males of many species increase their call repetition rate upon the approach of a female, and that males which have been removed from amplexus continue to call at a higher rate (Gerhardt, 1988). The normal call repetition rate is probably balanced against its cost in terms of energy expenditure (see Taigen & Wells, 1985) but males can "afford" to indulge in a higher rate for short periods of time. Call repetition rate has not been implicated in mate recognition.

Call duration is a temporal character which is fairly stable within species, which suggests that it may have a mate recognition function. Congeneric species often vary considerably in call duration. Three of the five species of *Hyla* listed by Gerhardt (1988) prefer calls of normal duration, but this preference is SPL dependent in two of the three. The remaining two species prefer longer calls. In comparing the call of *Hyperolius marmoratus* to those of two sympatric congeners, Dyson (1989) identified duration as the most important recognition character. In *Bombina bombina*, *B. orientalis*, and *Rana temporaria*, call duration is not used for the purpose of discrimination (Walkowiak & Brzoska, 1982; Walkowiak, 1988). Thus, in spite of its relative stability, call duration does not appear to play a significant role in mate recognition in most of the species investigated to date.

Gerhardt (1978a) showed that female *Hyla cinerea* can recognise a fine-temporal property of a synthetic call, viz: **waveform periodicity** , but only if the periodicity is reflected in the **amplitude-time envelope** of the call. Gerhardt presented females with two calls: one containing a 900 + 3000 Hz component and the other containing 900 + 2700 + 3000 Hz components. Both calls had a waveform periodicity of 300/s (the normal periodicityfor this species) but the latter call differed from the former in that the 2700 Hz and 3000 Hz components interacted to produce **beats** which were visible in the amplitude-time envelope of the call. He found that females preferred the calls containing beats, and also that they selected those which had a periodicity near the normal rate in preference to those with higher or lower rates.

Amplitude modulation of the call produces **pulses** of sound which are reflected in the amplitude-time envelope of the call. The pulses may correspond to the waveform periodicity of the call (ie. beats, sensu Gerhardt, 1978a) or each pulse may contain a number of waveforms. The presence or absence of pulses in the call may serve a recognition function: eg. *Hyla versicolor* will ignore a continuous, harmonically related tone, even if there is no alternative stimulus, but responds when the tone is amplitude modulated to form pulses (Gerhardt, 1978b). The calls of *H. gratiosa* and *H. cinerea* both have a pulsatile beginning which is essential for recognition in the former but not in the latter species (Gerhardt, 1981). In rapidly pulsed calls, sidebands are generated

in the frequency spectrum on either side of the dominant frequency band (see 1.4.2 above), but Gerhardt (1978a) has shown that the response to pulsed signals is based on recognition of the amplitude modulation of the call (ie. a temporal feature) rather than the spectral features (side bands) which it generates.

The rate at which pulses are produced is referred to as the **pulse rate**. This has been identified as an important mate recognition character in a number of species, eg: *Litoria verreauxi* and *L. ewingi* (Loftus-Hills & Littlejohn, 1971a), *Hyla regilla* and *H. cadaverina* (Straughan, 1975), *H. versicolor* and *H. chrysoscelis* (Gerhardt, 1978b, 1982), and *H. microcephala*, *H. ebraccata* and *H. phlebodes* (Schwartz, 1987). In all these cases the normal pulse rate was preferred to either higher or lower rates. **Pulse shape** can also be recognised: eg. *H. versicolor* females prefer calls with a long rise time over those with a short rise time (Gerhardt, 1988).

Rose & Capranica (1983, 1985) showed that in *Rana pipiens* the central auditory system responds to fine temporal properties such as amplitude modulation by way of synchronous discharges (by locking onto a particular phase of the wave), that they do not prefer a particular periodicity, and that the degree of synchronisation depends on the depth of modulation. Phase-locking is restricted to frequencies below 1 kHz (Narins & Hillery, 1983; Zakon & Wilczynski, 1988). Rose & Capranica (1985) showed that receptors in the basilar and the amphibian papillae of the inner ear are triggered by sinusoidally amplitude modulated signals ranging from 10 to 150 Hz, and that the basilar papilla is superior to the amphibian papilla in its ability to resolve temporal signals.

While the peripheral auditory system performs the initial analysis of the incoming signal in the spectrotemporal domain, it has been demonstrated that neurons in the frog's midbrain show the first clear temporal selectivity. Moreover, individual cells in the higher auditory centres exhibit a wide variety of response characteristics, confirming that temporal properties are analysed in detail in the central nervous system (Walkowiak, 1988). The existence of temporal encoding in the diencephalon and telencephalon has yet to be confirmed.

4.5.3 Spectral features.

The frequency spectrum represents another dimension within which sound energy may be partitioned in order to serve the recognition function of the advertisement call. In some species the energy is restricted to one spectral band, while other species have bimodal or more complex spectral patterns. Sometimes the frequency is temporally modulated: ie. it rises or falls between the beginning and the end of the call.

The components of the amphibian inner ear which are sensitive to frequency are the basilar papilla, which is sensitive to high frequencies (1.0 - 4.0 kHz), and the amphibian papilla, which is tuned to low- (< 100 Hz - 500 Hz) and mid- (500 Hz - 1.2 kHz) frequencies of the species audible range (Zakon & Wilczynski, 1988). For example the basilar papilla in *Acris crepitans* from New Jersey is most receptive to frequencies of 3500-3550Hz, while the amphibian papilla is most sensitive over the range 200-1000Hz (Capranica, 1977).

The receptors in the basilar papilla of a single individual are usually all tuned to about the same "best frequency", ie. a frequency at which sound, at a certain critical SPL, will elicit a response. The range of best frequencies for individuals of *Hyla crucifer* is about 100 Hz and that of *Eleutherodactylus coqui* is approximately 200Hz (Zakon & Wilczynski, 1988). The range of best frequencies of basilar papilla fibers usually corresponds to the dominant (emphasized) frequency of the advertisement call and, within the species, can vary according to size (Loftus-Hills, 1973), sex (Narins & Capranica, 1976) and population (Capranica et al., 1973).

Receptors in the basilar papilla exhibit a relatively broad, V-shaped tuning curve (x axis = frequency, y axis = SPL). The best frequency lies at the bottom of the curve and corresponds to the minimum SPL required to elicit a response in the receptor, while the arms indicate the frequencies at which the same receptor will respond at higher SPLs. Therefore, a low-intensity tone at best frequency and a high-intensity tone differing in frequency by "several hundred Hertz" are both capable of eliciting a response in the receptor (Zakon & Wilczynski, 1988:146). Because the receptors are tuned to a narrow range of best frequencies, and their response is dependent upon

both frequency and SPL, it is unlikely that the receptors of the basilar papilla are capable of distinguishing between sounds of different frequency. Zakon & Wilczynski therefore conclude that "good frequency resolution must not be possible in those species that detect the advertisement call solely with the basilar papilla". Species which emphasize a single frequency band lying in the range of sensitivity of the basilar papilla (ie. high frequency) are therefore likely to be less selective with regard to frequency than those whose dominant frequency band lies in the mid- to low frequency range. Examples of the former cited by Gerhardt (1988) are *Bufo calamita* (median spectral peak = 1.500Hz; Arak 1983b), *Acris crepitans* (mean spectral peak = 3679Hz; Nevo & Capranica, 1985) and *Hyla crucifer* (mean = 2800Hz; Doherty & Gerhardt, 1984).

Other significant characteristics of the basilar papilla are that the best frequency of the receptors does not shift with temperature, and that it is inversely related to body size, both between and within species. The negative correlation between best frequency and body size can be accounted for by the fact that the basilar papilla functions as a simple mechanical resonator in which the resonance is related to the mass of the tectorial membrane: ie. larger frogs have a heavier tectorial membrane which resonates at a lower frequency than that of smaller individuals (van Bergeijk & Witschi, 1957; Zakon & Wilczynski, 1988).

By contrast, receptors in the amphibian papilla are receptive to a range of frequencies and are not as broadly tuned as those of the basilar papilla (Feng et al., 1975; Lewis et al., 1982; Capranica & Moffat, 1983). The receptors are arranged tonotopically along the length of the papilla, with the low-frequency receptors located at the rostral end and the high-frequency receptors at the caudal end. Frequency analysis is thought to be facilitated by a wave travelling along the tectorial membrane, and there is some histological and physiological evidence to support this contention (Lewis & Leverenz, 1983; Hillery & Narins, 1984; Zakon & Wilczynski, 1988). This organ thus has a greater absolute sensitivity than the basilar papilla and is capable of true frequency discrimination (Fuzessery, 1988; Gerhardt, 1988; Zakon & Wilczynski, 1988).

In spite of these differences, behavioral studies involving *Hyla cinerea* (Gerhardt, 1987), *H. gratiosa* (Gerhardt, 1981) and *Physalaemus pustulosus* (Ryan, 1983b) found no significant difference in the ability of females to recognize frequency differences in both the high- and low-frequency ranges of the calls. Gerhardt (1988) suggested that this may be due to the effect of SPL on the function of the receptors in the respective organs: receptors in the amphibian papilla have a lower minimum intensity threshold (30 dB in hylids) than the basilar papilla (60 dB). A high intensity signal would stimulate receptors with a wide range of best frequencies in the amphibian papilla thereby masking its greater frequency selectivity. The selectivity of females should therefore be tested at different SPLs in order to accurately determine the frequency range over which phonotaxis occurs (Gerhardt,1988).

The best frequency range of the amphibian papilla varies between groups such as genera, but is fairly constant within them, while the best frequency range of the basilar papilla is species specific, corresponding to the dominant frequency of the advertisement call: this is an important indication of its basic function. It has also been observed that the frequency range of the amphibian papilla is similar in both sexes, and that this papilla exhibits temperature sensitivity (Moffat & Capranica, 1976; Zakon & Wilczynski, 1988; Stiebler & Rheins 1990).

Frequency modulation of the advertisement call has also been implicated in mate recognition, but only in cases where the frequency sweep falls within the range of both papillae or at least the amphibian papilla, eg. *Physalaemus pustulosus* (Ryan, 1983b) *Kassina senegalensis* and *K. maculata* (pers. comm. Capranica et al., reported in Zakon & Wilczynski, 1988). In cases where the frequency sweep falls within the range of the basilar papilla alone, individuals do not discriminate between a normal sweep and a reversed sweep:eg. *Hyperolius marmoratus* (pers. comm. P. Bishop, 1995).

Therefore, with regard to the significance of spectral features in mate recognition, advertisement calls may be divided into two groups:

a) Calls which excite both papillae: these may take the form of a broad frequency band, or they may consist of two or more separate bands, eg.*Hyla arenicola* and *H. femoralis* (Blair, 1958), *Rana catesbeiana* (Capranica ,1965) and *Hyla cinerea* (Gerhardt, 1974). Females are able to discriminate different frequencies in these calls and, in the case of *Physalaemus pustulosus*, were even able to discriminate between males, using spectral characteristics alone (Ryan, 1985).

b) Calls which excite only the basilar papilla: females show little preference for frequency as long as they fall within the frequency range of the basilar papilla (Wilczynski et al., 1984).

4.5.4 Synopsis.

Behavioral studies using synthetic calls have identified certain spectral and temporal cues that facilitate mate recognition in various species. It is unlikely that a single call character is responsible for recognition, but rather that a combination of characters is involved (Nevo & Capranica, 1985; Schwartz, 1986, 1987; Wells, 1988; Gerhardt & Doherty, 1988). For example, in *Hyla versicolor* , while pulse rate is of primary importance, call repetition rate, call duration, temperature and SPL all significantly affect the females response. Certain characters such as SPL and call repetition rate may alter or even negate preferences based on other characters (Gerhardt, 1988). Various combinations of call characters have been implicated in mate recognition, eg:dominant frequency and duration in *Microhyla carolinensis* (Blair, 1955,1958), pulse rate & pulse number in *Pseudacris* (Fouquette, 1975), pulse rate & duration in *Hyla chrysoscelis* and *H. versicolor* (Ralin, 1977), and pulse rate, duration and number & position of frequency bands in *Ptychadena* (Passmore, 1977, 1981).

The temporal characters most often implicated in recognition are the presence or absence of pulses and pulse rate, while the shape of the amplitude-time envelope and call duration are less frequently of importance. Call repetition rate does not appear to have a recognition function. The significance of the grouping of calls within a call bout (a feature of the calls of certain *Breviceps* species) has apparently not been investigated in the context of mate recognition.

With regard to spectral features, females generally prefer calls falling within the normal frequency range of their species, a phenomenon which has given rise to the concept of a sound window or matched spectral filter (Frishkopf et al., 1968; Capranica & Rose , 1983). However, these preferences are eliminated by small changes in SPL, and Gerhardt (1988, 1989), Fuzessery (1988) and others therefore argue that frequency discrimination is not important in the context of mate choice.

The perception that mate recognition systems are co-adapted or "co-evolved" (sensu Littlejohn, 1977:264), leads one to expect that the population mean of the the male's signal will correspond to the mean female receiver tolerance (Polakow et al., 1995). It is therefore interesting to note that the females of certain species of frogs do not respond preferentially to the population means of advertisement call components. For example, in *Hyperolius marmoratus*, females prefer calls with frequencies below the mean (Dyson & Passmore, 1988) and call rates above the mean (Passmore et al., 1992). Similarly, in an investigation of frequency tuning of the female's peripheral auditory system (Ryan et al., 1992) showed that in *Acris c. crepitans*, females preferred calls that had a frequency lower than the population mean.

It has also been observed that preferences exhibited in two-choice phonotaxis experiments do not necessarily operate in the more complex acoustic environment of a natural chorus, where the calls of numerous conspecific males approach a female from various directions and at different SPLs. For example, the ability of females to recognise small differences in frequency during two-choice experiments was greatly reduced when they were offered a choice of four calls (Gerhardt, 1982). Similarly, Dyson (1985) found that mating success was biased towards large males of *Hyperolius marmoratus* in low-density choruses, but that as male density increased, random mating occurred. Calls are usually alternated in phonotaxis experiments, whereas in large natural choruses they often overlap. Dyson & Passmore (1988) found that when *Hyperolius marmoratus* females were presented with alternating conspecific calls, they discriminated in favour of lower frequencies, but if the calls overlapped, females responded to the leading call, irrespective of frequency.

Nevertheless, the function and relative importance of individual call characters cannot be determined from field observation alone but need to be tested under controlled experimental conditions. Calls also contain characters which appear to be unimportant in intraspecific communication: these too may be identified in call-choice experiments. Examples of such characters in the calls of various *Hyla* species are illustrated by Gerhardt (1988:478).

Investigations into the function of an advertisement call must consider the possibility that the call may serve functions other than mate recognition "the multidimensional nature of acoustic signals is advantageous because different kinds of information can be encoded simultaneously" (Gerhardt, 1988:473). These additional functions may also be investigated and verified under natural and/or experimental conditions.

4.6 Variation in advertisement call parameters.

The advertisement call is clearly the fundamental constituent of the SMRS in most frog species and plays a principal cohesive role in frog populations. While the environment exerts selection pressure directly or indirectly (eg. by pleiotropy) on the advertisement call, the receiver (female) represents a selective force which tends to stabilise the call (Paterson, 1985, 1993a; Wells, 1988) Thus selection produces a co-adapted, sender-receiver system which is subject to minimal variation within the local population.

The evidence reviewed in 4.5 indicates that recognition is mediated by particular characteristics of the call. These specific characters are subjected to the stabilising effect of co-adaptation whereas other call characters are not (Lambert & Paterson, 1984). Thus, in the context of the Recognition Concept of species, the relative variability of call characters depends on whether or not they are involved in mate recognition. If this premise is correct then it logically follows that "features that show little variation within species are those most likely to be important for species recognition" (Wells, 1988:433; see also Schleidt, 1974; Narins & Capranica, 1977).

Variation of advertisement call parameters **within** populations is slight (Littlejohn & Martin, 1969; Littlejohn, 1968; Littlejohn et al., 1971) and has, in the case of spectral properties, been identified as a means whereby females can assess male fitness (eg. Ryan, 1990). However, **inter-population** comparisons have sometimes revealed substantial differences in call parameters implicated in mate recognition: this has led some authors to question the hypothetical stability of the SMRS.

The genus *Acris*, a North American taxon, has featured prominently in this debate. Capranica et al.(1973) found that populations of *Acris crepitans* from South Dakota (subspecies *blanchardi*) and New Jersey (subspecies *crepitans*) differ in temporal and spectral call characteristics. They suggested that the frequency selectivity of females is due to differences in the sensitivity of the basilar and amphibian papillae of the inner ear.

An in-depth, multidisciplinary study of *Acris c. crepitans*, *A.c. blanchardi*, and *A. gryllus* carried out by Nevo and Capranica (1985), revealed geographic variation in 16 spectral and temporal call variables within and between specie all of which are significantly correlated with morphological, ecological and geographical parameters. Multivariate analysis results in a clustering of the data which corresponds to the three biomes occupied by *Acris*: grassland (*A.c. blanchardi*), deciduous woodland (*A.c. crepitans*) and subtropical pineries (*A. gryllus*).

Phonotaxis experiments involving natural and synthetic calls and electrophysiological experiments which tested the females' frequency discrimination abilities show that females of *A.c. crepitans* discriminate against the calls of males from different populations of their own subspecies and that the level of discrimination increases with their geographic separation. The two subspecies, *crepitans* and *blanchardi* discriminate against one another completely, as do the two species, *crepitans* and *gryllus*. Both spectral and temporal call characters were found to be crucial cues for the females' discriminatory abilities. An interesting observation is that *A.c. blanchardi* display a far weaker degree of discrimination against *A. gryllus* than is the case with *A.c. crepitans*.

Ryan & Wilczynski (1988) investigated frequency discrimination in populations of *A.c. blanchardi* from Austin & Bastrop in central Texas, which are located within 65km of one another and occupy different habitats. They found that the dominant frequencies of the calls in the two populations differ by about 300 Hz and that in each population, the dominant frequency is closely matched to the best excitatory frequency of the basilar papilla. In phonotaxis experiments, females from Austin prefer the lower frequency calls of their own population to the higher frequency calls of the Bastrop males. The authors omitted to report on the phonotactic response of the Bastrop females to calls of Austin males. The data were adjusted to remove the effect of body size, and the results show that this factor is not responsible for the observed divergence in dominant frequency in the two populations. They concluded that divergence in the SMRS can take place in geographically proximate populations between which gene flow may conceivably be occurring.

The significance of the preference exhibited by the Austin females had to be re-evaluated when Ryan et al. (1992) found that in four phonotaxis experiments where females exhibited a preference, they always choose the lower-frequency calls. This explains why Austin females preferred their own calls (dominant frequency (df) = 3.5 kHz) to those of the Bastrop population (df = 3.8 kHz). However they also found that Bastrop females prefer calls equal in frequency to those of the Austin population. An examination of the frequency tuning of the female's peripheral auditory system showed that the basilar papilla is tuned lower than the mean call frequency of the male within each population. However, differences in dominant frequency in different populations are correlated with differences in auditory tuning: thus there is still evidence for population-based mating preferences. Another significant finding is that within a population large females prefer low-frequency calls while small females prefer calls with a higher frequency. The authors felt that this could reduce directional selection on the call and maintain variation in the male trait. They also suggested a possible reason for the mismatch between the dominant frequency of the call and the auditory tuning of the female, viz. that the call may be directed at both sexes, and that the dominant frequency of the call may therefore represent a compromise between the auditory tuning of both sexes.

The cause of inter-population variation in *Acris* calls has been attributed to environmental factors. Ryan et al. (1990) showed that the transmission efficiency of *Acris* calls is higher in open grassland (the habitat of *A.c. blanchardi*) than in forest (the habitat of *A.c. crepitans*). They found that the calls of *crepitans* and *blanchardi* transmit equally well in both habitats, but that the shorter, higher-pitched calls of *crepitans* exhibit less degradation in the forest habitat than those of *blanchardi*. They therefore reason that higher environmental selection pressure in the forest habitat has caused an adaptive shift in the structure of the *crepitans* call, while the grassland habitat has not exerted as great a selection pressure on the calls of *blanchardi*, which consequently have a longer duration and a lower dominant frequency. Ryan and Wilczynski (1991) investigated the correlation between call structure and habitat over a wider area and reported an east-west clinal variation in call characters. They suggest that call structure is associated with habitat type (ie. grassland or forest) and that the clinal nature of the variation is due to the passive effect of gene flow between populations in the zone of transition between habitat types. They claim that their findings are contrary to the RC prediction that strong stabilizing selection acts on species-specific mate recognition signals.

Paterson (1993a) questions the validity of this claim. Firstly, he points out that the subspecies studied were not delineated using RC criteria, and that the observed variation may in fact indicate that *crepitans* and *blanchardi* are actually separate genetical species. Secondly, he notes that the SMRS signals sampled were not postselection samples ie. they were male calls to which the female response had not been recorded. Therefore, some of the variation observed in the calls may not have elicited a response from the female and should not be included as part of the range in variation of the SMRS. He also queries methodological aspects which may have given rise to some of the observed variation, such as the use of air temperature instead of body temperature, and the significance of individual call parameters as opposed to the call as a whole.

As regards the occurrence of variation in SMRS characters, Paterson (1993a:219) states: "coadaptation imposes constraints on variation, leading to a degree of stability. Invariance is certainly not expected". He interprets the variation observed between

different populations of *A.c. blanchardi* as follows (p221): In vagile generalist species such as *Drosophila melanogaster*, the stability of the SMRS recorded in populations from around the world (Henderson and Lambert, 1982) is to be expected. *Acris crepitans*, on the other hand is not particularly vagile. "This would suggest that gene flow between the local populations is curtailed, facilitating local divergence due to the following plausible phenomena: founder effect, drift, adaptation". Thus a certain amount of variation may occur between populations of non-vagile species such as *Breviceps*, especially if they occupy a variety of habitats and are subjected to different environmental extremes.

4.7 Hybridization and advertisement calls.

The presence of hybrid swarms over a broad area of sympatry between *Breviceps mossambicus* and *B. adspersus*, has been reported by Poynton (1964, 1982) and Poynton & Broadley (1985). It is therefore necessary to briefly review some of the literature pertaining to hybridization in frogs.

Hybrids are the progeny of interbreeding individuals from populations that are genetically distinct in that they can be distinguished from one another by one or more characters (Littlejohn & Watson, 1985). Taxa that are broadly sympatric may produce isolated hybrids or, in cases of more extensive introgression between the parent populations, hybrid swarms, whereas interbreeding between parapatric or contiguously sympatric taxa results in hybrid swarms which are restricted to narrower zones (Mayr, 1963).

Hybrids exhibit various degrees of fertility and viability, which determine the structure and dynamics of hybrid zones. A cross is in one direction (eg. female x crossed with male y), may yield viable offspring, while the reciprocal cross (female y crossed with male x) does not (eg. *Scaphiopus*, Wasserman, 1957).

Two types of zone are recognised by Littlejohn & Watson (1985:87):

a) A **zone of "overlap with hybridization"** contains both parents and hybrids. If the hybrids are fertile and fully viable, the zone is transient and results in introgression between the two populations; if the hybrids are of inferior fitness a dynamic equilibrium develops, maintained by the immigration of individuals from the areas on either side of the zone. Zones of this type have been recorded between *Geocrinia laevis* and *G. victoriana* (Littlejohn & Watson, 1973, 1985) and between *Litoria ewingi* and *L. paraewingi* (Watson, 1972; Littlejohn, 1976; Littlejohn & Watson, 1985). While the maximum distance over which interactions took place (ie. the distance between pure parental populations) is relatively wide, most of the transition in call structure took place over a relatively short distance. It was also found that different characters vary in the width of their zones of replacement and exhibit different rates of change.

b) A **"true hybrid zone"** contains a predominance of hybrids which are fitter than their parents and it effectively separates the two parental populations. Zones of this type have been recorded between *Geocrinia laevis* and *G. victoriana* (Littlejohn & Watson, 1973, 1985)

Both of these types of zone occur within 6 km of one another along the interface between the two hybridising *Geocrinia* taxa (Littlejohn & Watson, 1985).

In structure, hybrid calls are usually intermediate between those of the two parental populations (Blair, 1958; Bogert, 1960) and some have a mosaic structure, ie. they contain elements characteristic of both parental species (Wasserman, 1957; Littlejohn et al., 1971; Littlejohn & Watson, 1973, 1976a, 1985; Gerhardt, 1983). Call characters in populations containing hybrids also exhibit a higher coefficient of variability than populations without hybrids (Littlejohn, 1972).

Zweifel (1968) found that in hybrids between *Bufo. a. americanus* and *B. woodhousii fowleri*, pulse rate is a better indicator of hybridization than call duration or morphology. While duration tended to be intermediate, its range in the hybrids overlaps the

ranges of the parent species. Some specimens which were identified as hybrids on the basis of pulse rate, cannot be distinguished morphologically from one of the parent species.

In *Geocrinia laevis* and *G. victoriana* the advertisement calls consist of a long, pulsed introductory note followed by a series of shorter, pulsed notes. The calls of the two species differ markedly in duration, pulse rate and in the repetition rate of the shorter repeated notes, but their respective ranges of dominant frequency and the number of pulses per repeated note overlap. In hybrids, four call parameters show significant variation: pulses per introductory note, duration of repeated notes, and pulse repetition rate of introductory and repeated notes (Littlejohn & Watson, 1973, 1985).

Gerhardt (1974) investigated three pairs of North American *Hyla* spp. and found that in 2 cases the hybrid calls are uniformly intermediate while in the 3rd case the hybrid call more closely resembled one of the parental species. Most of the parental species discriminate against the respective hybrid calls, while two species respond to hybrid calls presented without an alternative.

Doherty & Gerhardt (1983) showed that female hybrids of reciprocal crosses between *Hyla chrysoscelis* and *H. femoralis*, when presented with a choice of natural hybrid calls or the calls of the parental species, discriminate against *chrysoscelis* but not against *femoralis*. However they do discriminate against synthetic calls similar to those of *femoralis* in which the pulse rate is maintained at the mean value for that species and the total sound energy of the call is the same as that of the alternative call. They supported the idea (Alexander, 1962; Hoy et al., 1977) that common neural elements exist in both the sound producing systems of males and the sound recognition systems of females, ie. that the systems are genetically coupled. Evolution is seen as simultaneously modifying both the sender and receiver mechanisms in the same way, which would explain why hybrid females preferred the calls of hybrid males.

Littlejohn & Watson (1985:85) view hybrid zones as "critical stages in the dynamics of the speciation process", resulting in either "introgression, accelerated divergence

(character displacement) or stalemate". In the case of "true hybrid zones" Littlejohn & Watson (1973:284) contend that "the maintenance of distinctness of mating-call structures over extensive areas on either side of the interface in spite of presumably long periods of contact, support the recognition of these taxa as distinct species". However, in a later study (1976b) they showed that a natural hybrid mating call which contained elements characteristic of both parental species is attractive to females of both taxa. This raises the question: if the hybrid zone has been maintained for several thousand years (Littlejohn et al., 1971), why is it so narrow? The explanation suggested by these authors (1985:79) is that the zone "appears" to lie near an ecotone and they concluded that there was "strong selection against recombination products in areas away from the ecotone".

In terms of the Recognition Concept, on the other hand, hybridising populations are considered to be conspecific since they share a common mate recognition system (see 3.4.6 e). Hybrid zones are thus indicative of incomplete divergence of the SMRS, and are expected to result, ultimately, in complete introgression between the two populations.

5. Scope and objectives.

This thesis is principally concerned with the description and analysis of the advertisement calls of *Breviceps* species collected at a number of localities in the eastern part of southern Africa. The main objective is to use the advertisement call data to identify and describe the genetical species of *Breviceps* which occur at these localities, and relate them to existing taxonomic species and subspecies. It also aims to identify hybrid populations and undescribed taxa which may be present in the area sampled.

Diagnostic morphological characters of specimens referred to described species, are examined to determine whether the existing diagnoses are adequate or require revision. Other data pertaining to reproductive biology are recorded in an appendix to the main body of the thesis.

POPULATIONS SAMPLED AND METHODS USED

1. Populations sampled.

Specimens were collected from 62 populations at 57 localities in eastern South Africa, Swaziland, Mozambique and Zimbabwe. These localities are listed alphabetically in Appendix A, together with site and habitat details. Distribution maps showing the position of localities are contained in Appendix B.

2. Collecting period and method.

Data were collected during the breeding period which, in the species studied, lasts for 6 - 8 weeks following the first spring rains (Appendix D). Choruses develop shortly after rain begins to fall and may continue for several days and nights after a heavy downpour. Calling males were located by following the advertisement calls to source. Although the direction of the sound source can be judged accurately, the distance of the frog from the human observer cannot be determined without listening to the calling male from two or three different positions in order to pinpoint its exact location. The callsite is usually a shallow depression, well concealed beneath living vegetation or plant debris.

Recordings were made on standard magnetic tape cassettes using a Nakamichi CM550 portable tape recorder and a Nakamichi CM300 microphone. The microphone was placed directly in front of the male at a distance of 10 to 30 cm and three call bouts were recorded.

A BAT 12 Digital Thermometer accurate to 0.01°C was used to measure air temperature near the surface of the ground immediately after each recording was made. The cloacal temperature of many specimens was also measured but this was not always possible as some specimens eluded capture. Also, an accurate measure of cloacal temperature of very small specimens was difficult to obtain since their body temperature tended to rise while they were being handled.

Air and cloacal temperature are closely correlated: $r = 0.89$; $N = 16$; $p = < 0.001$ in the Pietersburg sample of *B.a. adspersus*, and $r = 0.92$, $p = <0.0001$, $N = 77$ in the northern Mozambique sample of *B. mossambicus*. A similar correlation of $r = 0.90$ was recorded for *Hyperolius marmoratus* by Passmore & Malherbe (1985). Air temperature was therefore used in the calculation of temperature correction factors.

Other data recorded included: date and time, a description of the callsite, distance between adjacent calling males, chorus intensity, weather conditions, a description of the habitat, and any other observation relevant to reproductive biology. Soil samples were collected at most of the sites sampled and analysed for their sand, clay and organic content. The precise position and altitude of each locality was determined from the topographical series, 1:50 000 South Africa, issued by the Government Printer, Pretoria.

Specimens were weighed in the field using a Sartorius portable balance accurate to 0.01g. They were then placed in a beaker containing a 15% aqueous solution of urethane (ethyl carbamate). Upon cessation of heartbeat, they were removed and colour print photographs were taken of the dorsal, ventral and lateral aspects of each specimen. Snout-vent length was measured using vernier calipers, and morphological examination was carried out under a stereo microscope. A 70% solution of ethyl alcohol was injected into the body cavity and each specimen was labelled and placed in a plastic screwtop bottle containing the same solution. The bottles were stored in closed cardboard boxes to avoid prolonged exposure to light. These will be deposited in the collections of the Transvaal Museum, Pretoria.

3. Measurement of advertisement calls.

Narrow (45Hz analysis filter) and wide band (300 Hz analysis filter) sonagrams of at least three advertisement calls of each specimen were made using a KAY 7029A sound spectrum analyser, scanning a range of 8kHz. Bout and call variables measured from these sonagrams were:

Bout variables :

bout duration (s) - from the beginning of the first to the end of the last call.

number of calls - within a single call bout.

call rate (min^{-1}) - average number of calls per minute

% grouped calls - number of grouped calls in a call bout taken as a percentage of the total number of calls in the bout.

Interval between calls (s) - from the beginning of a call to the beginning of the next call.

single-single - interval between two single calls

single-group - interval between a single call and a call group

group-group - interval between two call groups

in group - interval between two calls within a group

Call variables:

call duration (s) - from the beginning of the first to the end of the last pulse, or, in nonpulsatile calls, the entire call.

number of pulses - within a single call.

pulse rate (s^{-1}) - the time between the beginning of the first and the beginning of the last recognisable pulse divided by the number of pulses between these points.

dominant frequency (Hz) - the frequency measured at the centre of the darkest band of the narrowband sonagram.

sideband interval (Hz) - energy-free region between the central energy band and the sidebands flanking it on the narrowband spectrogram.

Bout duration and the interval between bouts were measured in the field or from the audiotapes by means of a digital stopwatch, and are accurate to 1 second. The interval between calls and call duration were measured in milliseconds, directly from the sonagram, using a transparent overlay provided by Kay Elemetrics Co. The overlay has graduations of 0.01 second (for sonagrams scanning a frequency range of 8kHz). Accuracy was enhanced by the use of a binocular microscope, but a margin of error of

up to 0.002 second may be expected. Frequency was measured from narrow band spectrograms (45Hz analysis filter) with frequency markers inserted at intervals of 1kHz. Measurements were made using a Zeiss zoom stereomicroscope fitted with an eyepiece graticule. When spanning two frequency markers, the smallest graduations on the graticule scale were 10Hz apart. Frequency was measured to 1Hz, but a margin of error of up to 2Hz may be expected.

4. Analysis of the data.

Statistical analysis of the data was performed using Statgraphics version 5.0. In addition to the mean, standard deviation and range, other standard statistics such as the median, interquartile range and standardised skewness were computed in order to show the biases in frequency distribution or central tendency of the data for each population sample. The median and interquartile range allow one to assess the extent to which the mean and range (max - min) have been influenced by extreme values, while standardised skewness values outside the range +2 to -2 indicate that the data may deviate significantly from a normal distribution. The coefficient of variation (expressed as a percentage) allows one to compare the amount of variance in variables that are measured in different units.

Pearson's correlation coefficients were calculated, and variables found to be strongly correlated with air temperature or body mass were adjusted to a common temperature or mass using the slope of the regression line (m) as a correction factor ($\Delta y = m \Delta x$).

5. Procedure followed when comparing populations:

5.1 Comparison of population samples in which the ranges in body mass or air temperature are congruent.

a) If the ranges of all measured call variables of one population sample fall within the ranges of the same variables in another population sample, or if the corresponding ranges overlap broadly the two populations are considered to be conspecific. In cor-

recting this data the correction factor of the larger sample (if > 100) is applied to both samples, or the two samples are combined and a common correction factor is applied.

b) If one or more variables occupy distinctly different ranges in two population samples, the possibility that they represent two species is considered.

c) In cases where two or more localities are relatively close together, the habitats are similar, and the call data are so similar that there is no reason to doubt that the samples are conspecific, the data are combined and treated as a single sample.

5.2 Comparison of population samples in which the ranges in body mass or air temperature are not congruent.

Such data may be corrected in cases where the ranges of body mass or air temperature of two populations are only slightly separated from one another, or if it is possible that the ranges might have overlapped had a larger sample been obtained. However, the correction of data which involves extrapolation far beyond the limits of the range of body mass and air temperature of a sample is considered unreliable because it involves the assumption that the correlation between the variables (ie. the slope of the regression line) does not change beyond those limits. At the present time there is no evidence to support this assumption except in the case of dominant frequency (Gerhardt, 1988). Data of this nature are therefore not corrected. Comparisons made between uncorrected data sets in which the ranges of body mass or air temperature are not congruent should be treated with circumspection.

Finally, it should be noted that when data has been corrected, the range of a variable decreases because the outliers are adjusted towards the mean: ie. the ranges reflected in the raw data are likely to be broader than those of corrected data. This should be borne in mind when comparing the ranges of raw data with those of adjusted data.

RESULTS & INTERPRETATION OF RESULTS

A. ADVERTISEMENT CALLS

1. Introduction.

Call discrimination experiments involving other frog species have demonstrated that the call functions as a mate recognition signal and can therefore be used to distinguish between genetical species (Introduction - 4.4 & 4.5). The 62 *Breviceps* populations sampled in this study form six distinct groups characterised by temporal differences in their advertisement calls, viz: the presence or absence of amplitude modulation (ie. pulsed vs. unpulsed calls), number of pulses and call duration. Five groups are assigned to described species while the sixth represents an undescribed species.

The assumption that these six groups represent genetical species is supported by the following considerations:

a) The temporal call characters which distinguish the groups from one another have a mate recognition function in a number of other species (see Introduction, 4.5.2).

b) The differences between the groups, in terms of these characters, are maintained in sympatry (eg. several localities sampled in this study).

c) The temporal call characters display a low coefficient of variation.

d) Five of the groups correspond to described species, some of which possess distinctive morphological characters (eg. toe length and dorsal skin ridges in *B. verrucosus*).

In grouping the data, two major divisions are recognised, viz: those which have pulsed calls and those which have unpulsed calls. Within each of these divisions populations are grouped according to the number of pulses in the call and call duration, respectively. This grouping is not intended to imply phylogenetic relationships.

In the case of pulsed calls, number of pulses is used in preference to call duration because the former is not correlated with temperature (Table 3): direct comparisons are possible without correcting the data. Fig. 7 & 8 illustrate the distribution of the data in respect of the number of pulses in the call, for the four species with pulsed calls.

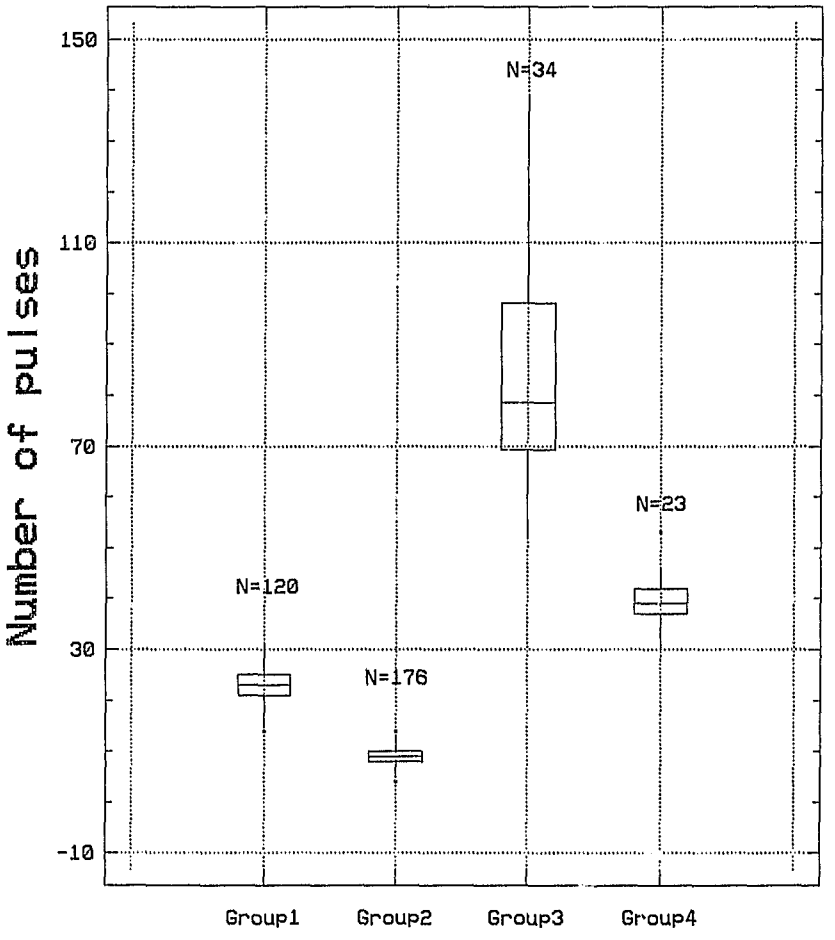

FIGURE 7. Box & whisker plots of pulse number for Groups 1-4. The plot divides the data into four areas of equal frequency and the median is represented by the horizontal line in the box. Samples from Localities 5, 7, 16, 17 & 35 are excluded.

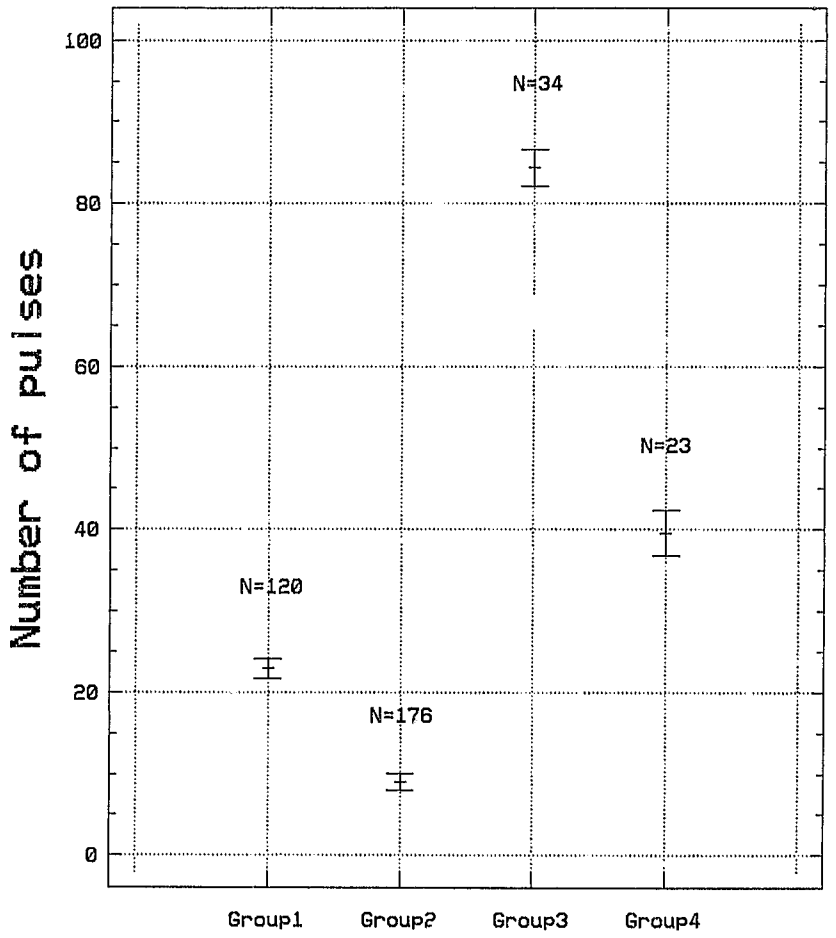

FIGURE 8. Means plot of pulse number for Groups 1-4. 95% confidence intervals about the means are indicated. Samples from Localities 5, 7, 16, 17 & 35 are excluded.

Grouping of the populations sampled:

Pulsatile calls:

Short calls: < 30 pulses; calls are occasionally or often grouped within the call bout.

Mean no. of pulses = 23 (range 14 - 31) - **Group 1**

Mean no. of pulses = 10 (range 4 - 18) - **Group 2**

Long calls: >30 pulses; calls are never grouped within the call bout.

Mean no. of pulses = 84 (range 52 - 139) - **Group 3**

Mean no. of pulses = 40 (range 38 - 53) - **Group 4**

Non-pulsatile calls:

Short calls: mean duration = 0.140s (range 0.11 - 0.16s); calls grouped within the call bout. - **Group 5**

Long calls: mean duration = 1.21s (range 0.78 - 1.98s); calls not grouped within the call bout. - **Group 6**

The advertisement call data from the populations assigned to these six groups are presented and discussed below. Other differences in call structure, which may or may not be implicated in mate recognition, are indicated and the homo/heterogeneity of the groups with regard to advertisement call structure is discussed. A description of the location, altitude, vegetation, soil and aspect of each locality is given in Appendix A.

2. Group 1 (*Breviceps adspersus* Peters 1882)

Most of the specimens assigned to this group possess the diagnostic morphological characters of *B.a. adspersus* recognised by Poynton & Broadley (1985). In some individuals the light paravertebral markings are entirely obscured by darker pigmentation (eg. Localities 3, 16, 21, 28, 38): these appear to represent the *Breviceps mossambicus* x *adspersus* hybrids defined by the above authors and by Lambiris

(1989a). However, with the exception of Loc.16 (Ingwavuma), the advertisement calls of these individuals do not reveal conclusive evidence of introgression and they are therefore assigned to *B.a. adspersus* (or *B.a. pentheri* in Eastern Cape material).

2.1 Advertisement calls of the Pietersburg population (Loc. 42).

2.1.1 Introduction.

Advertisement calls of 100 specimens were collected on 37 separate occasions at this locality between November 1984 and January 1993, at air temperatures ranging from 9.5 - 20.3 ° C. Chorus intensity was highest during and immediately after rain and slowly decreased over a period of several days in the absence of further precipitation.

Because of the relatively large size of this sample, it is used below to determine the degree to which advertisement call and call bout variables are correlated with air temperature and body mass. Call bout and call characters which are considered to have a mate recognition function are identified. Only these characters are subsequently used to describe and compare the other populations sampled in this study.

2.1.2 Structure and variation of call bouts.

A call bout consists of a series of similar calls emitted at the the rate of about 63 calls/min (over the duration of the entire bout). The interval between call bouts ranges from 1.5 to 10 min (mean = 3.7 min; N = 100). Call bouts follow one another at shorter intervals in high-intensity choruses than in low-intensity choruses.

Within a call bout, calls are usually emitted in call groups consisting of 2, 3 or more calls (Fig. 9). Approximately 83% of the calls emitted during a call bout are grouped in this way (Fig. 27b). The number of calls per group is higher during high-intensity choruses than in moderate or low intensity choruses, and single calls are usually restricted to the beginning and/or end of a bout. The percentage of single calls in the bout is higher during low-intensity choruses.

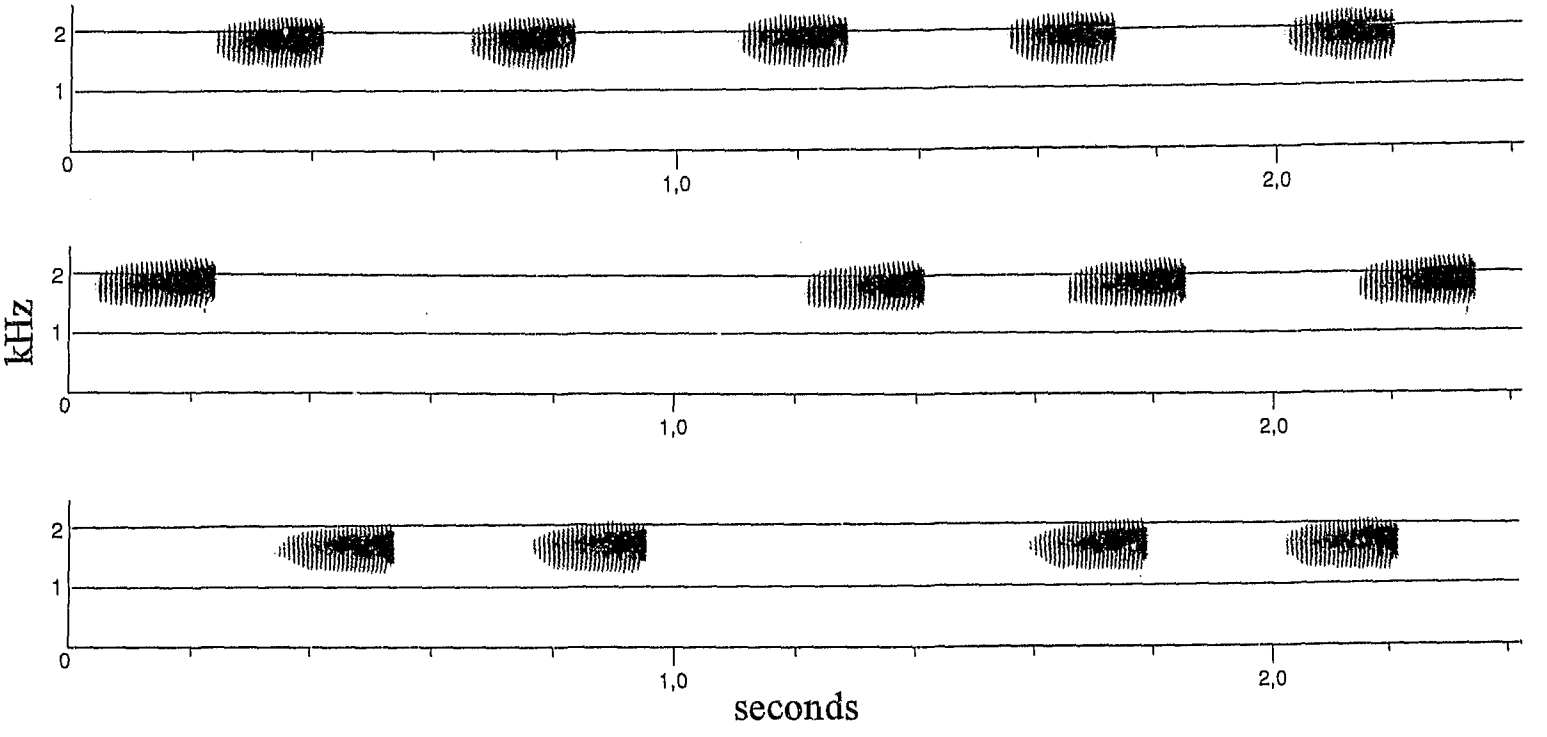

FIGURE 9. Call grouping within the call bout of *B.a. adspersus* from Loc.42 (Pietersburg)..

The call interval within a call group is considerably shorter than the interval between two single calls, two call groups or a single call and a call group (Table 1). Call bout characters which are significantly correlated with air temperature are call rate (r = 0.64), the interval between two call groups (r = -0.60), and the call interval within a group (r = -0.76; Table 3). The data do not reflect strong correlations between temporal bout variables and body mass.

Bout duration, the number of calls in the bout, call rate, the percentage of grouped calls and the interval between call groups, are all highly variable characters (Table 1). The call interval within groups has a relatively low coefficient of variation when corrected to 18° C (Table 4), indicating that this is a relatively stable call bout character.

2.1.3 Structure and variation of individual calls.

The call (or "note" sensu Duellman & Trueb 1985:89) consists of a short, pulsatile whistle which has a slow rise time and a rapid fall time (Fig. 10, WB). A typical call has a duration of 0.2 s and consists of about 24 pulses (Table 2). The pulses are equally spaced throughout the call except at the end, where the last two or three pulses are closer together. Single and grouped calls do not differ in structure.

An harmonic series is present, within which the fundamental frequency is dominant (mean = 1734 Hz). The frequency spectrum consists of a central, dominant frequency band flanked by discrete sidebands (Fig. 10, NB). As many as three pairs of sidebands may occur. Frequency modulation is often present in the form of a slight upward sweep from the beginning to the end of the call but sometimes the frequency sweeps downward slightly at the end of the call, or it may rise and fall slightly within a single call.

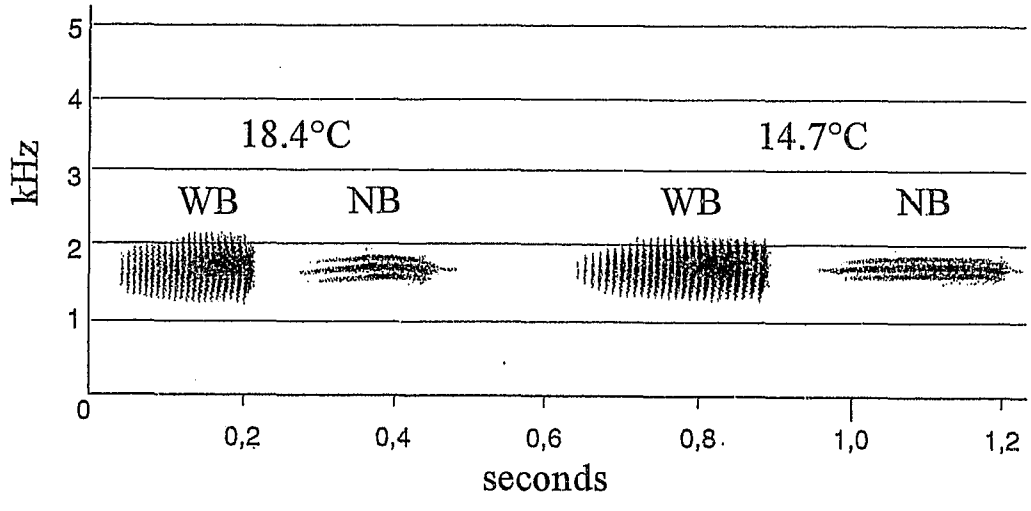

FIGURE 10. Wide (WB) and narrow band (NB) sonagrams of the advertisement calls of a single male of *B.a. adspersus* from Loc.42 (Pietersburg), recorded at different temperatures on the same night.

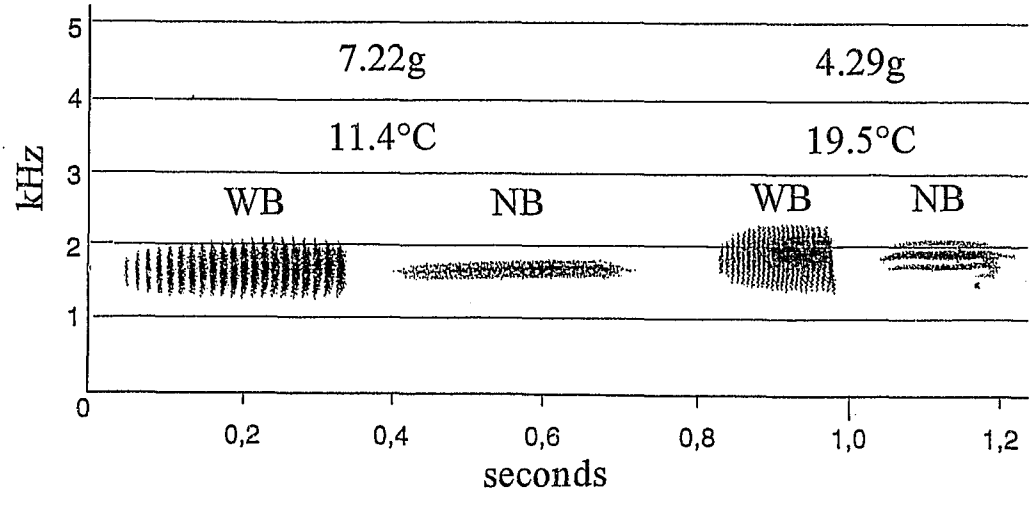

FIGURE 11. Wide (WB) and narrow band (NB) sonagrams of the advertisement calls of two different males of *B.a. adspersus* from Loc.42 (Pietersburg), illustrating the effect of temperature and body mass on call parameters.

TABLE 1. Summary statistics of call bout variables of a population of *B.a. adspersus* sampled at Loc.42 (Pietersburg). Air temperature range: 9.5 - 20.3°C.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Bout duration (s)	97	24.7	10.9	2.2	4.0	51.8	23.8	18.3	1.58	44.50
Number of calls	97	25	11.2	2.2	4	51	23	15	1.98	45.48
Call rate (min^{-1})	97	63	21.2	4.2	13	113	60	31	0.84	33.68
% grouped calls	97	79	23	5	0	100	85	20	-7.48	29.05
Call interval (s):										
single-single	8	1.742	0.399	0.277	1.260	2.290	1.761	0.743	-0.01	22.92
single-group	10	1.544	0.312	0.194	0.978	2.010	1.570	0.200	-0.59	20.22
group-group	61	1.215	0.386	0.960	0.579	2.210	1.179	0.542	1.18	31.78
in group	94	0.544	0.113	0.023	0.358	0.820	0.536	0.190	2.04	20.72

TABLE 2. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B.a. adspersus* sampled at Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Dom. frequency (Hz)	100	1734	91	18	1482	1939	1726	138	0.24	5.23
Sideband interval (Hz)	99	124	29	6	75	240	120	45	2.80	23.14
Call duration (s)	100	0.207	0.037	0.007	0.143	0.293	0.196	0.054	2.26	17.97
Number of pulses	100	24	2.5	0.5	17	31	23	3	2.11	10.38
Pulse rate (s^{-1})	100	116	21.8	4.3	71	165	114	34	0.11	18.80
Air temperature (°C)	100	15.7	2.8	0.6	9.5	20.3	16.0	4.6	-0.90	17.94
Body mass (g)	68	6.25	1.39	0.33	3.56	9.53	6.26	1.89	-0.87	22.24

TABLE 3. Pearson's correlation coefficients (r) calculated from linear regressions of call bout and advertisement call variables on air temperature and body mass of a population of *B.a. adspersus* sampled at Locality 42 (Pietersburg).

Variable	Air temperature (°C)			Body mass (g)		
	N	r	p	N	r	p
Bout duration (s)	96	-0.32	.002	66	0.13	.311
Number of calls	96	0.13	.214	66	-0.06	.644
Call rate (min^{-1})	96	0.64	<.001	66	-0.27	.027
% grouped calls	96	0.11	.278	66	-0.15	.223
Call interval:						
group-group	60	-0.60	<.001	46	0.48	<.001
within group	91	-0.76	<.001	61	0.35	.005
Call duration (s)	99	-0.74	<.001	67	0.30	.014
Number of pulses	99	0.35	<.001	67	-0.14	.251
Pulse rate (s^{-1})	99	0.84	<.001	67	-0.35	.003
Dominant frequency (Hz)	99	0.44	<.001	67	-0.63	<.001
Sideband interval (Hz)	98	0.72	<.001	67	-0.37	.002

TABLE 4. Summary statistics of call bout and advertisement call variables of a population of *B.a. adspersus* sampled at Loc.42 (Pietersburg), adjusted to a standard air temperature of 18°C (temporal variables) and body mass of 6g (dominant frequency only).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Dom. frequency (Hz)	68	1760	66	16	1621	1906	1763	93	0.49	3.77
Call duration (s)	100	0.185	0.025	0.005	0.126	0.251	0.183	0.035	0.51	13.53
Pulse rate (s^{-1})	100	131	11.8	2.4	94	156	132	19	-0.87	9.05
Call int. in group (s)	94	0.472	0.075	0.015	0.306	0.653	0.468	0.097	1.46	15.89

Of the temporal call variables, call duration and pulse rate are strongly correlated with air temperature ($r = -0.74$ & 0.84 respectively; Table 3, Fig.10&11), while the number of pulses is weakly correlated ($r = 0.35$). None of these characters is strongly correlated with body mass. With regard to the spectral call characters, dominant frequency is negatively correlated to body mass ($r = -0.63$; Table 3, Fig.11), while sideband interval is positively correlated to air temperature ($r = 0.72$;Table 3, Fig.11). The latter is to be expected since sideband interval is strongly correlated with pulse rate: $r = 0.89$ ($N = 99$; $p = <0.001$).

2.1.4 Variables used to compare the populations sampled in this study.

SMRS characters are less variable than other characters because of their co-adapted nature (Introduction, 3.4.6). Therefore call bout and call variables which have a low coefficient of variation are more likely to be involved in mate recognition than other, more variable characters.

Call bout variables: the **call interval within group** is the only call bout variable which, after temperature correction, shows a relatively low coefficient of variation (Table 4). However, it was observed in the field that some populations differ markedly in the **percentage of grouped calls** in a call bout, particularly during moderate or high intensity choruses. Therefore, in spite of its high coefficient of variation, this bout character is also used to describe and compare the populations sampled. The **number of calls in the call group** is another character which, in spite of its variability within populations, differs noticeably between populations.

Call variables: **dominant frequency** has the lowest coefficient of variation, followed by the **number of pulses, call duration** and **pulse rate** (Table 2). When the data are adjusted to a common body mass of 6g, in the case of dominant frequency, or to a common air temperature of 18° C, in the case of pulse rate and call duration, the coefficient of variation for each of these variables decreases substantially (Table 4). Of all adjusted call characters, dominant frequency and pulse rate show the lowest variability. These spectral and temporal variables have been shown to function in mate

recognition in a number of other frog species (Introduction, 4.5).

In frogs, sidebands in the frequency spectrum have not been specifically implicated in mate recognition. Since the distance between sidebands is strongly correlated with pulse rate (Fig.11), a change in one of these parameters results in a proportional change in the other. In this study populations are compared with regard to pulse rate, therefore a consideration of sideband interval is not deemed necessary.

Body mass and **air temperature** are also recorded because of their effect on certain call variables.

2.1.5 The application of correction factors to the data .

The correlation that exists between certain call variables and body mass or air temperature, complicates the comparison of samples in which the ranges of body mass or temperature differ. This problem can be overcome by correcting the data sets to a common body mass or air temperature (see Methods, 4), but fairly large samples are required in order to obtain accurate correction factors; this is shown in Table 5 below, in which **r** (correlation coefficient) and **m** (slope of the regression line) values calculated from the large Pietersburg sample of *B. adspersus* are compared with values calculated from smaller portions of the same sample.

The Pietersburg (Dalmada) sample comprises the call data of 100 specimens collected from a single population over an extended period, arranged in chronological order of collection. The sample is divided in the following way:

ADSDAL. represents the entire sample of 100 specimens.
DAL 1 - 5 each comprise 20 different specimens of ADSDAL.
DAL 6 - 8 each comprise 33 different specimens of ADSDAL.
DAL 9 - 10 each comprise 50 different specimens of ADSDAL.
DAL 11 - 12 comprise the top 75 and bottom 75 specimens of ADSDAL respectively ie. 50 specimens are the same and 25 differ in each sample.

TABLE 5. Variation in the correlation coefficient (r) and the slope of the regression line (m), calculated by linear regression of **pulse rate** on **air temperature** for different portions of the same population sample (ADSDAL) of *B.a. adspersus* from Loc.42 (Pietersburg):

Sample code	Specimen numbers	N	r	p	m	Temperature range
ADSDAL	1 - 100	100	**0.840**	<.0001	**6.48**	10.8 (9.5 - 20.3)
DAL 1	1 - 20	20	0.721	<.0001	4.68	7.5 (9.5 - 17.0)
DAL 2	21 - 40	20	0.782	<.0001	8.38	5.6 (11.4 - 17.0)
DAL 3	41 - 60	20	0.903	<.0001	6.83	7.9 (11.5 - 19.4)
DAL 4	61 - 80	20	0.186	.43	2.38	3.0 (17.3 - 20.3)
DAL 5	81 - 100	20	0.905	<.0001	7.80	5.8 (14.0 - 19.8)
DAL 6	1 - 33	33	0.726	<.0001	5.92	7.5 (9.5 - 17.0)
DAL 7	34 - 66	33	0.889	<.0001	7.60	8.2 (11.4 - 19.6)
DAL 8	67 - 99	33	0.841	<.0001	8.08	6.3 (14.0 - 20.3)
DAL 9	1 - 50	50	0.650	<.0001	5.69	8.6 (9.5 - 18.1)
DAL 10	51 - 100	50	0.827	<.0001	8.16	6.3 (14.0 - 20.3)
DAL 11	1 - 75	75	0.845	<.0001	6.79	10.2 (9.5 - 19.7)
DAL 12	26 - 100	75	0.867	<.0001	7.63	8.9 (11.4 - 20.3)

Table 5 demonstrates that:

1. There is considerable variation in **r** and **m**, especially in the smaller samples, to the extent that in the case of sample DAL 4 the two variables are not significantly

correlated. The low correlation coefficient in this sample may be due to the fact that the temperature range is very narrow and that 60% of the data points occupy only 25% of the temperature range, ie. the spread of the data is extremely uneven. The samples of 50 data items (DAL 9&10) also differ substantially in their **r** and **m** values.

2. Samples which have similar **r** values may have substantially different **m** values (eg. DAL 1&2 or DAL 3&5).

3. Although samples collected over a very narrow temperature range (eg. DAL 4) may not reveal a significant correlation, they sometimes yield a higher **r** value than samples taken over a wider temperature range, eg: DAL 10 vs 9 or DAL 5 vs 1, or DAL 5 vs ADSDAL. Furthermore, samples taken over a similar temperature range (eg. DAL 2&5) may differ in their **r** values.

4. The values of **r** & **m** for DAL 11&12 are fairly close to those of ADSDAL, but this may be partly due to the fact that 50 of the 75 specimens in DAL 11&12 are shared.

Correction factors calculated from small samples (<20) using the method of simple linear regression employed above, are therefore less reliable than those derived from larger samples (>50).

2.2 **Other populations in the Northern Province and Mpumalanga.**

2.2.1 Loc. 14 (Houtbosdorp 1), Loc.28 (Lydenburg 2) & Loc. 57 (White River)

The raw data presented in Table 6 show that the advertisement calls of the single specimens from these three localities do not differ significantly from those of the Pietersburg population: the only variable which falls outside the range of the Pietersburg sample is the number of pulses in the Lydenburg sample. Therefore the data are adjusted using correction factors derived from the Pietersburg sample.

The adjusted call durations of the Lydenburg and White River specimens are relatively low but not significantly different from the Pietersburg population. In the case of the sample from White River, the chorus intensity was very low ; the predominance of

single calls in low intensity choruses is not unusual in *B.a. adspersus*. These three populations are therefore assigned to *B.a. adspersus*.

2.2.2 Loc. 34 (Mica) & Loc. 13 (Hoedspruit)

These populations are located close to one another in similar habitats, and no inter-population differences are evident in the advertisement call data. The samples are therefore combined in Table 7 . The ranges in air temperature and body mass of this sample and those of the Pietersburg sample are congruent, and the call bout and call variables of the two samples overlap extensively: therefore correction factors calculated from the Pietersburg sample were used to adjust the raw data to a common body mass and temperature.

The ranges of the adjusted variables of these populations fall well within, or broadly overlap those of the Pietersburg population (Tables 1 & 2). The populations from Mica and Hoedspruit are therefore assigned to *B.a. adspersus*.

2.2.3 Loc. 21 (Komatipoort 1).

Although the temperature ranges of the two samples are not congruent, the median air temperature of the Komatipoort sample is only 2.4°C above the maximum for the Pietersburg sample. Therefore, for the purpose of comparison, the Pietersburg sample has been adjusted to the median body mass and air temperature of the Komatipoort sample (Table 8).

The Komatipoort sample displays a wide range in dominant frequency which reaches a maximum 271Hz higher than that of the Pietersburg sample. This maximum is due to a single small specimen, the remainder falling within the dominant frequency range of the adjusted Pietersburg sample.

The call duration lies at the lower limit of the range of the adjusted Pietersburg sample as does the number of pulses: 16 - 19, as opposed to 17 - 31 in the unadjusted Pieters-

burg sample (this variable is not correlated with temperature). Call interval within call groups also falls within the range of the adjusted Pietersburg sample. Another similarity between these two samples lies in the percentage of grouped calls and the fact that 33% of the call groups in the Komatipoort sample consist of three or more calls (up to seven calls in a group).

Pulse rate is the only variable which does not overlap but the respective sample ranges are separated by only a narrow margin of 18 pulses per second (8% of the mean pulse rate). The mean pulse rates of the two populations differ by a factor of 1.3. Littlejohn (1969) and Gerhardt (1988) have noted that the pulse rates of sympatric cognate species typically differ by a factor of two or more. Thus the difference in pulse rates of these two populations appears to be too small to suggest a difference at the species level. A larger sample may help to clarify this question. This population is therefore provisionally referred to *B.a. adspersus*.

2.2.4 Aural records: Loc. 29 (Manzini) & Loc. 55 (Trichardtsdal)

Strong choruses were heard at these localities but recordings were not obtained. The calls were grouped in a way typical of the Pietersburg population, and are provisionally referred to *B.a. adspersus* pending further investigation. At both localities two species occur in parapatry: one at a lower elevation (these populations) and the other (see Group 2) on the adjacent mountain slopes. A specimen collected at Manzini has an unusual dorsal pattern of light spots which is also present in one of the Komatipoort specimens.

TABLE 6. Advertisement call variables, air temperature and body mass of samples comprising single individuals of *B.a. adspersus* from populations at Loc.14 (Houtbosdorp 1), Loc.28 (Lydenburg 2) and Loc.57 (White River).

Variable	Loc. 42		Loc. 14	Loc. 28	Loc. 57
	mean	range			
Raw data:					
Dom. frequency (Hz)	1734	1482 - 1939	1766	1553	1785
Call duration (s)	0.207	0.143 - 0.293	0.169	0.144	0.144
Number of pulses	23.8	17 - 31	21	16	21
Pulse rate (s^{-1})	116	71 - 165	122	107	141
% grouped calls	79	0 - 100	91	75	0
Call int. in group (s)	0.544	0.358 - 0.820	0.485	0.386	--
Air temperature (°C)	15.7	9.5 - 20.3	16.2	15.6	16.4
Body mass (g)	6.25	3.56 - 9.53	5.85	9.87	--
Data adjusted to 6g and 18°C: *					
Dom. frequency (Hz)	1760	1621 - 1906	1760	1704	--
Call duration (s)	0.185	0.126 - 0.251	0.152	0.121	0.129
Pulse rate (s^{-1})	131	94 - 156	133	123	151
Call int. in group	0.473	0.306 - 0.653	0.431	0.332	--

* Correction factors for the Pietersburg sample (Loc. 42) are applied throughout.

TABLE 7. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B.a. adspersus* sampled at Loc.34 (Mica) and Loc.13 (Hoedspruit).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	10	1779	74	46	1630	1857	1795	70	-1.51	4.16
Call duration (s)	10	0.161	0.030	0.019	0.128	0.221	0.153	0.04	1.45	18.84
Number of pulses	10	21	1.9	1.2	19	26	21	1	2.29	9.38
Pulse rate (s^{-})	10	130	13.6	8.4	110	146	132	24	-0.64	10.43
% grouped calls	10	72	32	20	0	100	81	24	-2.01	44.78
C.I. within group (s)	9	0.386	0.059	0.039	0.319	0.490	0.374	0.084	0.73	15.39
Air temperature (°C)	10	17.8	2.5	1.5	14.1	20.2	19.4	4	-0.86	13.90
Body mass (g)	8	7.26	1.04	0.72	5.82	8.94	7.40	1.41	0.04	14.28
Data adjusted to 6g and 18°C: *										
Dom. frequency (Hz)	8	1820	88	61	1676	1924	1842	139	-0.50	4.83
Call duration (s)	10	0.159	0.018	0.011	0.129	0.184	0.163	0.027	-0.48	10.98
Pulse rate (s^{-})	10	131	9.9	6.1	121	151	129	13	1.14	7.54
C.I. within group	9	0.386	0.031	0.020	0.337	0.422	0.387	0.050	-0.38	7.99

* Correction factors for Pietersburg sample (Loc. 42) applied throughout.

TABLE 8. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B.a. adspersus* sampled at Loc.21 (Komatipoort 1), and of the adjusted sample from Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	4	1926	194	191	1780	2197	1864	282	1.06	10.09
Call duration (s)	4	0.079	0.005	0.005	0.074	0.083	0.079	0.009	0	6.62
Number of pulses	4	17	1.3	1.2	16	19	17	1.5	2.29	9.38
Pulse rate (s^{-})	4	212	9.7	9.5	205	226	208	11	1.53	4.60
% grouped calls	4	63	15	15	53	85	57	17	1.57	23.79
Call Int. in group (s)	4	0.300	0.048	0.047	0.273	0.374	0.281	0.052	1.60	15.94
Air temperature (°C)	4	22.7	0.3	0.3	22.3	23.0	22.7	0.5	-0.35	1.32
Body mass (g)	4	5.06	1.49	1.46	2.91	6.36	5.49	1.81	-1.24	29.51
Pietersburg sample adjusted to 5.49g and 22.7°C:										
Dom. frequency (Hz)	68	1780			1641	1926	1783			
Call duration (s)	100	0.139			0.080	0.205	0.137			
Pulse rate (s^{-})	100	161			125	187	162			
Call int. in group	94	0.369			0.203	0.550	0.365			

2.3 Populations in Zimbabwe.

2.3.1 Loc.3 (Burchenough Bridge) & Loc.38 (Mutare 1).

The samples from these two populations have been combined because of their proximity to one another and the similarities in the structure of their advertisement calls. The air temperature at Burchenough Bridge was 4 - 7°C higher than at Mutare 1, and consequently the calls from the former locality have a shorter duration and a higher pulse rate.

The samples differ slightly from the Pietersburg sample with regard to call duration and the number of pulses in the call, but correction of the Pietersburg data to the median body mass and air temperature of the Zimbabwe sample reduces the disparity in call duration (Table 9). The ranges of all variables are congruent or overlap: therefore these populations from Zimbabwe are assigned to *B.a. adspersus.*

2.4 Populations in Kwazulu/Natal.

2.4.1 Loc.16 (Ingwavuma), Loc .49 (Sihangwane 4), & Loc.50 (Sihangwane 5)

These samples have been combined because of their proximity to one another (<15km) and similarities in habitat, size and call structure.

At Loc. 16 (Ingwavuma) the advertisement calls range from short grouped calls to long single calls, while at the other localities only the latter were recorded. The two call types recorded at Loc. 16 were produced by different individuals participating in the same chorus, at the same time and at the same air temperature. This variability is reflected in the unusually large coefficients of variation for call duration and number of pulses (Table 10). This sample is compared directly with the Pietersburg sample because the medians and ranges in body mass and air temperature of the two samples overlap broadly and correction of the data is therefore unnecessary. The variability of

dominant frequency, pulse rate and call interval within the call group, is similar to that of the unadjusted sample from Pietersburg.

The dominant frequency ranges of the two samples overlap by 62%, with the Zululand sample extending 181Hz beyond the maximum of the Pietersburg sample. A similar relationship exists in the number of pulses in the calls. The range in call duration in the Zululand sample is double that of the Pietersburg sample, which is contained entirely within the range of the former. The percentage of grouped calls is lower in the Zululand sample because of the presence of individuals giving the longer, single calls. The ranges in pulse rate and in the call interval within call groups are very closely matched in the two samples.

There is no consistent difference which separates this Zululand sample from the Pietersburg sample, and this population is therefore provisionally referred to *B.a. adspersus*. However, the unusually large coefficients of variation in call duration, pulse number and the unusually long calls emitted by some individuals invite further investigation.

2.5 Populations in the Eastern Cape Province.

2.5.1 Loc. 7 (Grahamstown) & Loc. 41 (Paterson).

Grahamstown is presumed to be the type locality of *B.a. pentheri* (Poynton 1964). This sample includes one specimen from Paterson, 53km from Grahamstown. To facilitate a more accurate comparison, the Pietersburg sample is adjusted to the median body mass and air temperature of the Grahamstown sample (Table 11).

The dominant frequency ranges of the two samples overlap broadly and the range in pulse rate is also congruent. The ranges in call duration overlap slightly, with the Grahamstown calls tending to be shorter. The ranges in the number of pulses meet but do not overlap (Fig.32). Another difference between the populations is that the Grahamstown individuals seldom group their calls: this may be due to the fact that the sample

was collected over a relatively low air temperature range.

The differences between the two samples are not of sufficient magnitude to justify recognising *pentheri* as a separate species. The Grahamstown sample is therefore referred to *B.a. pentheri* (but see Discussion, 2.1.2).

2.5.2 Loc. 1 (Addo) & Loc. 20 (King William's Town)

These specimens from the Eastern Cape Province were collected from weak choruses. Table 12 shows that the Addo calls lie well within the ranges of call variables of the Pietersburg sample. The cloacal temperature of the Addo specimen was not recorded but was probably well below air temperature as it was calling from a depression in cool, damp soil, in full shade beneath succulent vegetation. The specimens from King William's Town (not collected) were calling weakly from well concealed positions in grass. Their calls are possibly shorter than the normal chorus call. All of these specimens are provisionally referred to *B.a. pentheri*.

TABLE 9. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B.a. adspersus* sampled at Loc.3 (Burchenough Bridge) and Loc.38 (Mutare 1) and of the adjusted sample from Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	7	1730	81	60	1630	1880	1710	94	1.03	4.66
Call duration (s)	7	0.119	0.021	0.015	0.090	0.148	0.126	0.035	-0.34	17.22
Number of pulses	7	15	1.4	1.0	14	18	15	2	1.05	9.06
Pulse rate (s^{-1})	7	129	22.5	16.6	109	166	116	41	0.97	17.38
% grouped calls	7	61	39	29	8	98	80	71	-0.46	63.12
Call int. in group (s)	6	0.369	0.046	0.037	0.306	0.426	0.363	0.077	0.06	12.40
Air temperature (°C)	7	18.4	2.9	2.1	16.0	23.1	16.9	5.6	1.22	15.64
Body mass (g)	7	7.56	1.15	0.85	5.80	8.92	8.08	2.09	-0.78	15.16
Pietersburg sample adjusted to 8.08g and 16.9°C:										
Dom. frequency (Hz)	68	1679			1540	1825	1682			
Call duration (s)	100	0.196			0.137	0.262	0.194			
Pulse rate (s^{-1})	100	124			87	149	125			
Call int. in group	94	0.505			0.339	0.686	0.501			

TABLE 10 . Summary statistics of advertisement call variables, air temperature and body mass of a population of *B.a. adspersus* sampled at Loc.16 (Ingwavuma), Loc.49 (Sihangwane 4), and Loc.50 (Sihangwane 5), and of the sample fromLoc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	19	1830	105	47	1657	2120	1793	159	1.99	5.74
Call duration (s)	19	0.259	0.097	0.044	0.099	0.398	0.287	0.176	-1.0	37.58
Number of pulses	19	27	7.3	3.3	16	39	27	11	-0.33	27.22
Pulse rate (s^{-1})	19	107	23.6	10.6	84	153	97	46	1.49	21.99
% grouped calls	19	30	38	17	0	97	6	73	1.41	126.15
Call int. in group (s)	10	0.532	0.118	0.073	0.392	0.741	0.494	0.193	0.69	22.17
Air temperature (°C)	19	16.9	1.6	0.7	14.6	21.1	16.5	0.8	3.12	9.46
Body mass (g)	19	5.83	1.33	0.60	3.96	9.08	5.83	1.86	1.24	22.83
Pietersburg sample (raw data):										
Dom. frequency (Hz)	100	1734	91	18	1482	1939	1726	138	0.24	5.23
Call duration (s)	100	0.207	0.037	0.007	0.143	0.293	0.196	0.054	2.26	17.97
Number of pulses	100	24	2.5	0.5	17	31	23	3	2.11	10.38
Pulse rate (s^{-1})	100	116	21.8	4.3	71	165	114	34	0.11	18.80
% grouped calls	97	79.0	23.0	4.6	0	100	85.3	20.4	-7.48	29.05
Call int. in group (s)	94	0.544	0.113	0.023	0.358	0.820	0.536	0.190	2.04	20.72
Air temperature (°C)	100	15.7	2.8	0.6	9.5	20.3	16.0	4.6	-0.90	17.94
Body mass (g)	68	6.25	1.39	0.33	3.56	9.53	6.26	1.89	-0.87	22.24

TABLE 11. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B.a. pentheri* sampled at Loc.7 (Grahamstown) and Loc.41 (Paterson), compared with the adjusted sample from Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	11	1909	108	64	1780	2050	1953	218	-0.15	5.64
Call duration (s)	11	0.136	0.025	0.015	0.098	0.178	0.129	0.036	0.66	18 33
Number of pulses	11	14	1.5	0.9	12	17	14	1	1.11	10.88
Pulse rate (s^{-1})	11	98	11.1	6.5	75	112	100	19	-0.96	11.33
% grouped calls	11	2	4	3	0	13	0	0	2.98	230.07
Air temperature (°C)	11	14.4	1.2	0.7	12.3	15.7	15.0	1.6	-1.34	8.03
Body mass (g)	11	4.38	1.45	0.86	2.50	7.61	3.9	1.64	1.26	32.98
Pietersburg sample adjusted to 3.9g and 15°C:										
Dom. frequency (Hz)	68	1842			1703	1991	1845			
Call duration (s)	100	0.214			0.155	0.280	0.212			
Pulse rate (s^{-1})	100	112			75	137	113			

TABLE 12. Advertisement call variables, air temperature and body mass of individuals of *B.a. pentheri* from Loc.1 (Addo) and Loc.20 (King William's Town), compared with the adjusted sample from Loc.42 (Pietersburg).

Variable	Locality 42		Loc. 1	Loc. 20	
	mean	range	No. 67	No. 422	No. 423
Raw data:					
Dom. frequency (Hz)	1734	1482 - 1939	1610	1765	1750
Call duration (s)	0.207	0.143 - 0.293	0.160	0.096	0.107
Number of pulses	23.8	17 - 31	17	12	12
Pulse rate (s^{-1})	116	71 - 165	101	107	111
% grouped calls	79	0 - 100	0	0	0
Air temperature (°C)	15.7	9.5 - 20.3	22	16.5	15.6
Body mass (g)	6.25	3.56 - 9.53	8.76	--	--
Pietersburg sample: body mass adjusted to 8.76g:					
Dom. frequency (Hz)	1653	1514 - 1799			

3. Group 2 (*Breviceps mossambicus* Peters 1854)

3.1 Introduction.

The populations assigned to this species are located in two widely separated areas, viz: northern populations from Mozambique Island (the type locality) and the adjacent mainland, and southern populations from Maputo, coastal Kwazulu/Natal, and the eastern escarpment of South Africa and Swaziland. Populations in central Mozambique were not sampled due to political unrest in the area when this study was carried out.

The specimens from northern Mozambique conform morphologically to the description of *B. mossambicus* Peters by Poynton & Broadley (1985), but those from southern Mozambique, Swaziland and South Africa possess light markings dorsally, a feature used by the above authors as a diagnostic character for *B.a. adspersus*. In some southern specimens the gular patch is clearly divided in the midline, while in others it is uniformly darkened as is the case in the northern material. In spite of the morphological differences, the southern material is assigned to *B. mossambicus* because of the similarities in the advertisement calls of the two groups (see 3.8, below).

3.2 Advertisement calls of a population from Mozambique Island (Loc 36).

3.2.1 Introduction.

This island is one of Peters' two type localities for *B. mossambicus*. It is approximately 3km long and 800m wide and is connected to the mainland by a bridge 3.5km long. Although the island is densely populated and little unoccupied space remains, *B. mossambicus* occurs in a public park adjacent to the Fortress at the northern end of the island, and in a cemetery at the southern end. The emergence of these frogs in large numbers following rain, as described by Peters (1882:117): "während des Regens in ungeheurer Zahl aus der Erde hervorkam", was observed in the park at 05:00 on 5.12.1994 following heavy rain. As many as 12 frogs per m^2 were moving about on the surface, and groups of 2-4 individuals were observed feeding on worker termites

at emergence holes in the soil. No calling was heard at this time. Advertisement calls of 58 specimens were collected at night, between 30.11.94 and 6.12.94, at air temperatures ranging from 24.8 - 27.1°C.

3.2.2 Structure and variation of call bouts

The call bout lasts for up to 30 seconds and consists of 9 - 39 calls emitted at the mean rate of 106 calls per minute (Table 13). On average, only 17% of the calls are grouped: in a sample of 68 bouts from 58 different males, 83% of the calls are single, 16% are in groups of two and 1% form groups of three calls. This contrasts strongly with *B.a. adspersus* from Pietersburg, where 83% of the calls are grouped and 43% are emitted in groups of three or more (Fig. 27b). As is the case in *B.a. adspersus*, the interval between two calls within a call group is considerably shorter than the interval between two single calls, two call groups or a single call and a call group (Table 13).

Owing to the narrow temperature range over which the calls were collected, the data do not yield significant correlations between temperature and call bout variables. No significant correlation exists between body mass and any call bout variable.

3.2.3 Structure and variation of individual calls

The call consists of a very short pulsatile chirp which has a rapid rise and fall time (Fig.12 & 13). A typical call has a duration of 0.05 seconds and consists of about 10 pulses produced at a rate of 192 pulses per second (Fig. 12, WB & Fig. 13; Table 14). The pulses are equally spaced throughout the call, except at the end, where the last two or three pulses are closer together.

The calls are not frequency modulated. An harmonic series is present, and the fundamental frequency is dominant (mean = 1792 Hz). The call and its harmonics have as many as three discrete sidebands on either side of the central, dominant frequency band (Figure 12, NB).

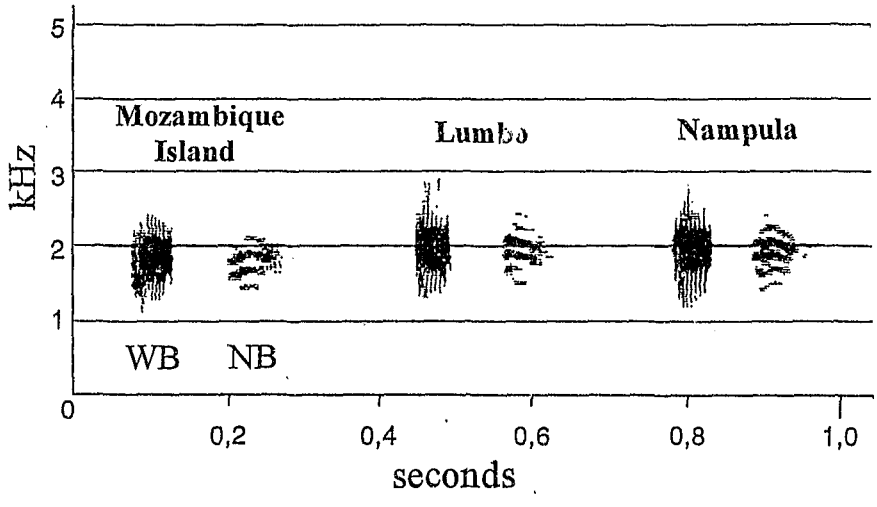

FIGURE 12. Wide and narrow band sonagrams of the advertisement calls of *B. mossambicus* from Loc.36 (Mozambique Island), Loc.26 (Lumbo) & Loc.40 (Nampula).

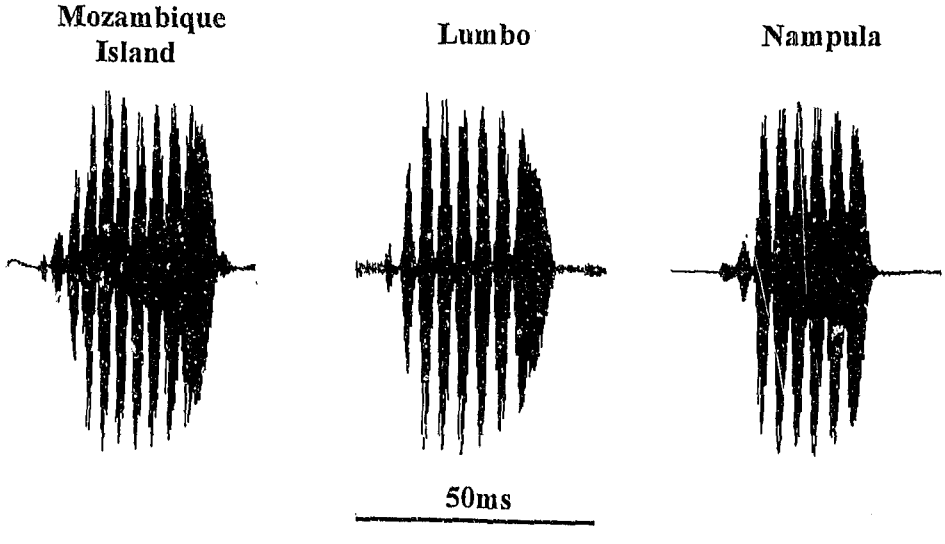

FIGURE 13. Oscillograms of the advertisement calls of *B. mossambicus* from Loc.36 (Mozambique Island), Loc.26 (Lumbo) & Loc.40 (Nampula).

TABLE 13. Summary statistics of call bout variables of a population of *B. mossambicus* sampled at Loc.36 (Mozambique Island). Air temperature range: 24.8 - 27.9°C.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Bout duration (s)	68	11.6	5.3	1.3	3.7	29.4	9.8	6.4	4.33	45.87
Number of calls	68	19	6.8	1.6	9	39	18	10	2.77	36.18
Call rate (min^{-1})	68	106	30.4	7.2	55	195	102	42	1.91	28.87
% grouped calls	68	17	25.3	5.9	0	96	0	34	4.79	149.36
Call interval (s):										
single-single	54	0.699	0.179	0.047	0.396	1.170	0.680	0.209	1.73	25.53
single-group	14	0.537	0.092	0.047	0.410	0.720	0.520	0.219	0.94	17.07
group-group	5	0.459	0.030	0.026	0.418	0.490	0.470	0.040	-0.60	6.49
in group	13	0.269	0.065	0.035	0.202	0.405	0.250	0.064	1.76	23.04

TABLE 14. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B. mossambicus* sampled at Loc.36 (Mozambique Island), compared with the sample from Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	58	1792	80	21	1600	1937	1792	120	-0.60	4.49
Call duration (s)	58	0.050	0.007	0.002	0.036	0.079	0.049	0.006	3.46	14.77
Number of pulses	58	10	1.2	0.3	7	13	9	1	1.14	12.94
Pulse rate (s^{-1})	55	192	14.8	3.9	152	229	192	18	-1.15	7.71
% grouped calls	68	17	25	6	0	96	0	34	4.79	149.36
Call int. in group (s)	13	0.268	0.065	0.035	0.202	0.405	0.250	0.604	1.76	24.03
Air temperature (°C)	58	26.9	0.8	0.2	24.8	27.9	27.1	1.3	-1.84	2.99
Body mass (g)	53	8.63	1.21	0.33	5.77	11.46	8.71	1.37	0.09	13.99
Pietersburg sample - variables adjusted to 8.71g and 27.1°C:										
Dom. frequency (Hz)	68	1655			1516	1801	1658			
Call duration (s)	100	0.096			0.037	0.163	0.095			
Pulse rate (s^{-1})	100	190			153	215	191			
Call int. in group	94	0.200			0.034	0.381	0.196			
Variables not adjusted:										
Number of pulses	100	24			17	31	23			
% grouped calls	97	79			0	100	85.3			

Because of the narrow temperature range, these data do not yield significant correlations between call variables and air temperature; the correlation between body mass and dominant frequency is relatively low ($r=-0.42$, $p=.001$, $N=53$).

3.2.4 Differences between the advertisement calls of *B. mossambicus* (Island) and *B.a. adspersus* (Pietersburg).

Because these species have sometimes been considered to be conspecific, it is appropriate at this point to establish whether their recognition as two separate species can be justified on the basis of advertisement call structure.

In Table 14, certain call bout and call variables of the Pietersburg sample of *B.a. adspersus* have been adjusted to the median air temperature and body mass of the Island sample of *B. mossambicus*. However, since the ranges in air temperature of the two samples are not congruent, the corrected temporal variables should be treated with circumspection.

Differences between the two samples in respect of call bout characters such as the % grouped calls and the number of calls within the groups have been noted (3.2.2).

With regard to call characters, there is a broad overlap in the range of dominant frequency in the two samples. Correction of the mean call duration of *B.a. adspersus* reduces it from .207s to .096s, which is still roughly twice the mean call duration of *B. mossambicus*. However the ranges of call duration in the two samples overlap almost entirely. Correction of the Pietersburg pulse rate data results in a very close correspondence between the two samples in terms of the mean, median and range. While call duration and pulse rate are strongly correlated with air temperature (Table 3: $r=-0.74$ and $r=0.84$ respectively) the number of pulses per call is not ($r=0.35$): i.e. as air temperature rises, call duration decreases and pulse rate increases, but the number of pulses does not change. Thus another significant difference is that *B.a. adspersus* calls contain, on average, twice as many pulses as *B. mossambicus* calls and their ranges in respect of this call character do not overlap.

Essentially the advertisement calls of the *B. mossambicus* (Island) population are similar to those of the *B.a. adspersus* (Pietersburg) population in their ranges of dominant frequency, pulse rate and in the call interval within groups. They differ in duration, number of pulses and in the percentage of grouped calls. The nature and extent of these differences indicate that the populations represent different genetical species, ie. the retention of the taxa *B.a. adspersus* and *B. mossambicus* is supported by the data.

3.3 Populations from mainland Mozambique.

3.3.1 Loc. 26 (Lumbo) & Loc. 40 (Nampula).

Although these localities are separated by 125km, the samples are combined (Table 15) because the means and ranges of all call variables coincide (Lumbo: N = 5; Nampula: N = 20). Most mainland specimens are much darker dorsally than those from the Island (Fig.3) and their mean mass is nearly half that of the Island sample. One adult male weighed 1.73g, which is well within the size range of the material from southern Mozambique and coastal Kwazulu/Natal (see Table 17).

When the Island data are corrected to the median body mass of the mainland sample, the dominant frequencies of the calls correspond closely (Table 15). Temporal call variables are not strongly correlated with air temperature in either sample, probably because the calls were recorded over a very narrow temperature range: thus it was not possible to apply a temperature correction factor to the data. However, the mean air temperatures of the samples differ by less than 4°C, and if it were possible to correct the data, the small existing differences between the samples in respect of the means and medians of pulse rate and call interval, would be reduced rather than increased.

The very strong similarity between the advertisement calls of the Island and mainland populations clearly indicate that they are are conspecific (Tables 14 & 15, Fig. 12 & 13). These data are therefore combined in Table 16, for comparison with populations in southern Mozambique and Kwazulu/Natal.

TABLE 15. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.26 (Lumbo) and Loc.40 (Nampula), compared with the sample from Loc.36 (Mozambique Island) .

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	25	1935	96	38	1780	2193	1937	94	1.05	4.94
Call duration (s)	25	0.051	0.006	0.003	0.040	0.065	0.051	0.011	0.06	12.35
Number of pulses	25	9	0.9	0.4	7	11	9	1	-0.47	10.49
Pulse rate (s^{-1})	25	174	8.9	3.5	160	198	174	6	1.30	5.08
% grouped calls	33	7	13	4	0	44	0	8	4.70	180.15
Call int. in group (s)	2	0.273	0.033	0.045	0.250	0.296	0.273	0.046	-32768	11.92
Air temperature (°C)	25	23.3	0.6	0.2	22.5	24.8	23.2	0.6	2.04	2.34
Body mass (g)	24	4.68	1.36	0.54	1.73	7.78	4.75	1.81	0.25	28.99
Mozambique Island sample (Loc. 36) adjusted to 4.75g.*										
Dom. frequency (Hz)	53	1924	73	20	1790	2073	1923	109	-0.21	3.82

* Correction factor derived from combined mainland & Island samples (r = -0.70, m = -34.19)

TABLE 16. Summary statistics of advertisement call variables, air temperature and body mass of all populations of *Breviceps mossambicus* from mainland northern Mozambique and Mozambique Island.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	83	1835	107	23	1600	2193	1830	145	1.61	5.84
Call duration (s)	83	0.050	0.007	0.001	0.036	0.079	0.050	0.007	3.02	14.08
Number of pulses	83	9	1.2	0.3	7	13	9	1	1.24	12.30
Pulse rate (s^{-1})	80	187	15.6	3.4	152	229	187	23	0.48	8.34
% grouped calls	83	8	16	3	0	76	0	8	8.58	203.49
Call int. in group (s)	15	0.270	0.060	0.031	0.202	0.405	0.250	0.77	1.89	22.44
Air temperature (°C)	83	25.8	1.8	0.4	22.5	27.9	26.4	3.8	-2.12	7.09
Body mass (g)	77	7.40	2.22	0.49	1.73	11.46	8.14	3.32	-1.93	30.06

3.4 Populations from southern Mozambique and coastal KwaZulu/Natal.

These populations are first compared with one another and with populations from the eastern escarpment of southern Africa. Their affinities with populations in northern Mozambique is discussed in Section 3.8.

3.4.1 Loc. 30 (Maputo), Loc. 23 (Lake Kosi), Loc. 46, & 47 (Sihangwane 1 & 2)

The data of two specimens from Maputo, one specimen from Lake Kosi (115km to the south), and eight specimens from Sihangwane 1 & 2 (38km west of Lake Kosi; Fig.17), are combined because of their close similarity with regard to call structure, size, appearance and habitat (Table 17).

These populations are characterised by a short, high-pitched pulsatile call (Fig.14) which is easily distinguished aurally from those of *B.a. adspersus* populations occurring in the same area (ie. Localities 16, 49 & 50). None of the specimens in this sample grouped its calls. All call variables show a low coefficient of variation.

3.4.2 Localities 51 & 52 (St. Lucia 1 & 2).

These two localities are 14km apart and the samples are combined because of their overall similarity. The sample (Table 18, Fig.14 & 17) can be compared directly with the previous one (Table 17) because the means and medians of body weight and air temperature are very similar. A small percentage of the calls were grouped, cf. the previous sample, but there is close correspondence between all other call variables. The two samples are therefore considered to be conspecific.

3.4.3 Locality 37 (Mtunzini).

This larger sample of 19 specimens (Table 19, Fig.14 & 17) can also be compared directly with the previous two samples. It differs slightly in having a higher percentage of grouped calls but in all other respects it matches them closely. It is clearly conspe-

cific with those samples.

3.4.4 Locality 6 (Eshowe).

This locality is situated 29km inland of Mtunzini, at a higher altitude, and has a darker, more loamy soil. The sample of calls of six specimens (Table 20, Fig.14 & 17) resembles the coastal population (Table 19) closely. The means and medians of the call variables correspond well and the ranges overlap broadly. The samples from Eshowe are therefore considered to be conspecific with those from Mtunzini.

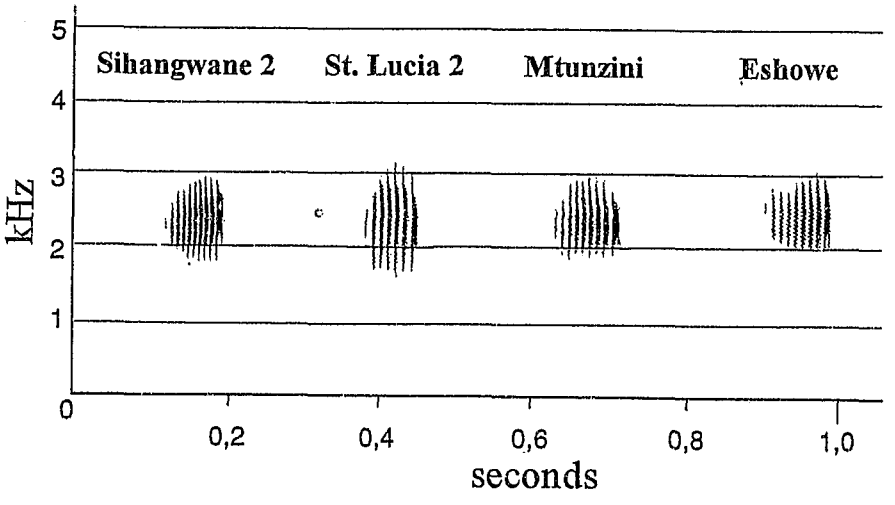

FIGURE 14. Wide band sonagrams of the advertisement calls of *B. mossambicus* from four populations in coastal KwaZulu/Natal.

TABLE 17. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.30 (Maputo), Loc.23 (Lake Kosi) and Loc.46 & 47 (Sihangwane 1 & 2).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	11	2507	124	73	2345	2710	2513	240	0.01	4.94
Call duration (s)	11	0.075	0.006	0.004	0.065	0.085	0.075	0.007	-0.35	8.04
Number of pulses	11	11	1.3	0.7	10	14	11	1	1.98	11.18
Pulse rate (s^{-1})	11	138	15.6	9.2	126	180	132	12	3.10	11.29
Air temperature (°C)	10	21.7	3.1	1.9	19.8	27.5	20.4	0.4	2.27	14.22
Body mass (g)	9	2.20	0.81	0.53	1.47	4.15	2.20	0.50	2.41	36.72

TABLE 18. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.51 (St Lucia 1) and Loc.52 (St Lucia 2).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	8	2484	82	57	2377	2608	2458	113	0.81	3.28
Call duration (s)	8	0.079	0.011	0.008	0.065	0.093	0.079	0.022	-0.06	14.13
Number of pulses	8	9	0.6	0.4	8	10	9	1	-0.08	7.02
Pulse rate (s^{-1})	8	104	12.5	8.7	88	128	101	15	1.05	12.03
% grouped calls	8	2	3	2	0	7	0	2.5	1.84	188.56
Air temperature (°C)	8	19.9	2.6	1.8	16.9	24	20.2	4.4	0.24	12.93
Body mass (g)	5	1.85	0.43	0.38	1.43	2.42	1.83	0.67	0.33	23.26

TABLE 19. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B. mossambicus* sampled at Loc.37 (Mtunzini).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	19	2487	68	31	2293	2563	2508	101	-2.54	2.74
Call duration (s)	19	0.080	0.016	0.007	0.046	0.104	0.083	0.011	-1.57	19.67
Number of pulses	19	10	1.5	0.7	7	12	10	2	-1.40	14.91
Pulse rate (s^{-1})	19	119	11.6	5.2	107	149	118	12	2.64	9.71
% grouped calls	19	36	27	12	0	75	36	54	-0.33	72.69
Call int. in group (s)	15	0.464	0.063	0.031	0.380	0.620	0.440	0.072	1.88	13.67
Air temperature (°C)	19	19.9	2.5	1.1	17.7	25.8	18.6	2.3	2.84	12.79
Body mass (g)	16	1.71	0.28	0.14	1.22	2.32	1.75	0.39	0.29	16.29

TABLE 20. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B. mossambicus* sampled at Loc.6 (Eshowe).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile r[illegible]	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	6	2539	64	51	2453	2645	2542	50	0.61	2.51
Call duration (s)	6	0.086	0.017	0.013	0.064	0.102	0.089	0.034	-0.44	19.53
Number of pulses	6	10	2.1	1.7	6	12	11	2	-1.34	21.73
Pulse rate (s^{-1})	6	106	19.3	15.4	82	130	99	34	0.48	18.23
Air temperature (°C)	6	17.3	6.1	4.9	13.9	29.5	14.9	2.6	2.31	35.08
Body mass (g)	5	1.87	0.43	0.38	1.46	2.58	1.70	0.22	1.37	22.95

3.4.5 Loc. 17 (Jozini 1) & Loc. 19 (Jozini 3).

These samples are combined in Table 21 because of the proximity of the populations to one another (23km) and the similarity in advertisement call structure, size and habitat. The advertisement call structure of this and the following two samples are illustrated in Fig.15. The Jozini call sample differs from previous samples (3.4.1 - 3.4.4) in having a slightly longer duration and a greater % grouped calls. It is therefore necessary to compare it with *B.a. adspersus* in order to determine whether or not its affinities lie with that species.

The specimens from these localities are relatively small compared to the Pietersburg sample which ranges from 3.56 - 9.53g; median, 6.26g. The Pietersburg sample is therefore adjusted to the median mass of the Jozini sample . After this adjustment, the ranges in dominant frequency of the two samples overlap very slightly with the mean of the Jozini sample lying 300Hz higher than that of the Pietersburg sample.

The mean call duration of the Jozini sample is less than half that of the adjusted Pietersburg sample and their ranges do not overlap. The Jozini advertisement calls have fewer pulses and there is only a slight overlap in the ranges of this variable. The ranges in pulse rate and the call interval within groups overlap broadly or are congruent in both samples. The mean % grouped calls in the Jozini sample is close to that of the Pietersburg population.

Thus, in respect of call duration, number of pulses and dominant frequency, the Jozini sample appears to be more closely related to the populations 3.4.1 - 3.4.4 above (eg. Table 17) than to *B.a. adspersus* and is therefore regarded as conspecific with those populations.

3.4.6 Loc. 35 (Mkuze).

This sample is compared with *B.a. adspersus* from Pietersburg (Table 22), and illustrated in Fig.15. The range in body mass lies within that of the Pietersburg sample, but

the air temperature mean and maximum are considerably higher. The Pietersburg sample has therefore been adjusted to the median air temperature and body mass of the Mkuze sample.

While resembling the Pietersburg sample in terms of the % grouped calls and the range in pulse rate, the ranges of several other call variables in this sample, viz: dominant frequency, call duration and the number of pulses do not overlap at all. Thus, in terms of call structure this population resembles the previous sample from Jozini more closely than *B.a. adspersus*, and is considered to be conspecific with the former.

3.4.7 Loc 5. (Durban).

In the past, specimens from Durban have been referred to *B.a. adspersus* (eg. Poynton 1964, Poynton & Pritchard 1976). In the present study only two specimens were collected at this locality but it is clear from Table 23 & Fig.15, that their calls differ from the adjusted Pietersburg sample of *B.a. adspersus* calls in the same ways as the populations sampled at Jozini and Mkuze. Thus they are also considered to be conspecific with the latter populations.

3.4.8 Advertisement call summary statistics of the southern, coastal populations of *B. mossambicus*.

The data from these populations (3.4.1 - 3.4.7) are combined in Table 24 for comparison with population samples from the eastern escarpment of South Africa and Swaziland (3.6).

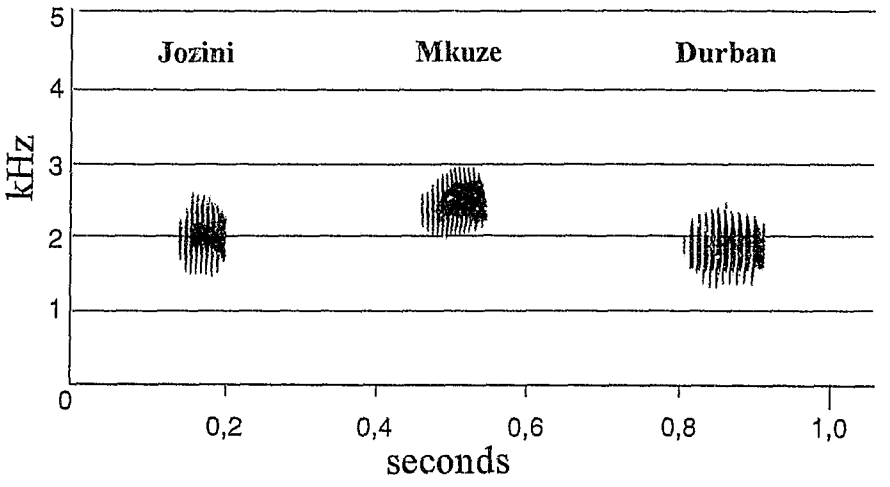

FIGURE 15. Wide band sonagrams of the advertisement calls of *B. mossambicus* from Loc. 17 (Jozini 1), Loc. 35, (Mkuze) and Loc.5, (Durban).

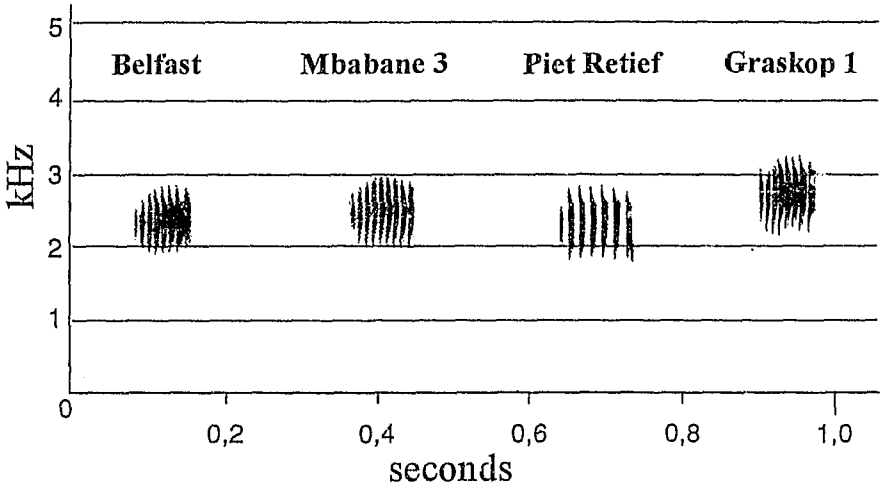

FIGURE 16. Wide band sonagrams of the advertisement calls of *B. mossambicus* from Loc.2 (Belfast), Loc.33 (Mbabane 3), Loc.43 (Piet Retief), Loc.8 (Graskop 1).

TABLE 21. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.17 (Jozini 1) and Loc.19 (Jozini 3), and of the adjusted sample from Loc.42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	9	2242	186	121	1974	2560	2198	193	0.19	8.28
Call duration (s)	9	0.077	0.019	0.012	0.053	0.104	0.071	0.029	0.05	24.14
Number of pulses	9	13	3.0	0.18	9	18	12	3	0.99	22.21
Pulse rate (s^{-1})	9	148	10.5	6.9	135	164	145	17	0.44	7.11
% grouped calls	9	74	18	12	38	100	74	11	-0.49	24.97
Call int. in group (s)	9	0.384	0.042	0.027	0.328	0.470	0.370	0.041	1.12	10.88
Air temperature (°C)	9	17.9	0.6	0.4	17.2	19.2	18	0.4	0.74	3.41
Body mass (g)	8	2.84	0.68	0.47	2.27	4.31	2.60	0.75	1.92	23.90
Pietersburg sample adjusted to 2.6g and 18°C:										
Dom. frequency (Hz)	68	1892			1753	2038	1895			
Call duration (s)	100	0.185			0.126	0.251	0.183			
Pulse rate (s^{-1})	100	131			94	156	132			
Call int. in group	94	0.472			0.306	0.653	0.468			
Variables not adjusted:										
Number of pulses	100	24			17	31	23			
% grouped calls	97	79			0	100	85.3			

TABLE 22. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B. mossambicus* sample at Locality 35 (Mkuze), and of the adjusted sample from Locality 42 (Pietersburg).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	16	2383	94	46	2205	2524	2394	117	-0.74	3.92
Call duration (s)	16	0.076	0.012	0.024	0.051	0.097	0.077	0.016	-0.83	15.95
Number of pulses	16	13	2.1	1.1	9	17	13	3	-0.08	16.67
Pulse rate (s^{-1})	16	161	16.0	7.8	143	202	157	21	1.83	9.93
% grouped calls	16	80	20	10	30	98	89	26	-2.18	25.31
C.I. within group (s)	16	0.316	0.047	0.024	0.240	0.403	0.319	0.082	0.07	15.00
Air temperature (°C)	16	19.8	2.7	1.3	15.0	25.8	19.7	2.4	0.23	13.42
Body mass (g)	13	4.96	0.59	0.32	4.26	6.27	4.78	0.43	1.57	12.01
Pietersburg sample adjusted to 4.78g and 19.7°C :										
Dom. frequency (Hz)	68	1807			1669	1954	1811			
Call duration (s)	100	0.169			0.110	0.235	0.167			
Pulse rate (s^{-1})	100	142			105	167	143			
C.I. within group	94	0.421			0.255	0.602	0.417			
Variables not adjusted:										
Number of pulses	100	24			17	31	23			
% grouped calls	97	79			0	100	85.3			

TABLE 23. Advertisement call variables, air temperature and body mass of two specimens of *B. mossambicus* from Loc.5 (Durban), compared with the sample of *B.a. adspersus* from Loc.42 (Pietersburg) adjusted to the mean mass and temperature of these specimens.

Variable	Locality 42		Locality 5	
	mean	range	No. 261	No. 262
Raw data:				
Dom. frequency (Hz)	1734	1482 - 1939	1863	2211
Call duration (s)	0.207	0.143 - 0.293	0.094	0.099
Number of pulses	23.8	17 - 31	10	12
Pulse rate (s^{-1})	116	71 - 165	111	114
% grouped calls	79	0 - 100	17	70
Call int. in group (s)	0.544	0.358 - 0.820	0.541	0.531
Air temperature (°C)	15.7	9.5 - 20.3	17.0	16.3
Body mass (g)	6.25	3.56 - 9.53	5.55	4.01
Pietersburg sample adjusted to 4.8g and 16.7°C:				
Dom. frequency (Hz)	1807	1668 - 1953		
Call duration (s)	0.198	0.139 - 0.264		
Pulse rate (s^{-1})	123	86 - 148		
Call int. in group	0.512	0.345 - 0.692		

TABLE 24. Summary statistics of advertisement call variables, air temperature and body mass of all samples of *B. mossambicus* from southern Mozambique and coastal KwaZulu/Natal.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	71	2427	154	36	1863	2710	2450	164	-4.66	6.33
Call duration (s)	71	0.079	0.014	0.003	0.046	0.104	0.079	0.02	-0.68	17.46
Number of pulses	71	11	2.2	0.5	6	18	11	2	-2.26	20.04
Pulse rate (s^{-1})	71	132	24.8	5.8	82	202	130	37	1.78	18.77
% grouped calls	55	35	34	9	0	100	33	67	0.88	95.47
Call int. in group (s)	42	0.394	0.088	0.027	0.24	0.62	0.385	0.111	1.09	22.31
Air temperature (°C)	70	19.6	3.1	0.72	13.9	29.5	19.3	2.7	3.48	15.80
Body mass (g)	58	2.8	1.43	0.37	1.22	6.27	2.25	2.55	2.80	50.90

3.5 Populations from the escarpment of Swaziland, northern KwaZulu/Natal Mpumalanga and the Northern Transvaal Province.

3.5.1 Introduction.

These populations occupy the slopes and crest of the eastern escarpment, from which areas specimens have, in the past, been referred to *B.a. pentheri*. The habitat here differs markedly from that of the coastal plain. The soil is loamy and often stony, and the dominant vegetation types are highland sourveld, mountain sourveld or sandy highveld. The altitude ranges from 950m to 2200m above sea level and minimum temperatures are more severe than along the coast: snowfalls are sometimes experienced in winter. Precipitation is higher here than it is in habitats occupied by *B.a. adspersus* further north and in the Lowveld to the east and northeast.

Owing to the small sample sizes, reliable correction factors could not be calculated, and comparisons are therefore based on raw data.

3.5.2 Loc. 2 (Belfast) & Loc 27 (Lydenburg 1)

The Belfast and Lydenburg localities are 80km apart. Their altitudes range from 1850 - 2200m above sea level, and the habitats are very similar. The Belfast sample (N = 10) shows the widest range in mass (1.71 - 4.96g) while the Lydenburg sample (N = 4; Fig. 17) lies just below the lower end of the range (1.21 - 1.47g). The samples overlap in respect of dominant frequency, call duration, pulse rate and pulse number, and correction of the data to a common mass and temperature would have the effect of shifting the means of these characters closer together, thus broadening the region of overlap. The samples are therefore combined in Table 25.

The advertisement call is a short, high-pitched, pulsatile chirp, usually emitted singly but sometimes in groups of two or three, especially during strong choruses (Fig. 16). The calls of these escarpment populations (3.5.2-3.5.6) are easily distinguishable, aurally, from parapatric populations of *B.a. adspersus*, which gives longer, lower-

pitched calls that are usually emitted in larger groups. Areas in which the two species occur in parapatry are Lydenburg 2 (Loc. 28), Trichardtsdal (Loc.55), and between Mbabane 2 (Loc.32) and Manzini.

3.5.3 Loc. 31, 32 & 33 (Mbabane 1, 2 & 3)

These Swaziland localities lie > 800m above sea level on the grassy, wet, eastern slopes of the escarpment. The samples are combined because they do not differ markedly from one another in any respect (Table 26, Fig. 16).

The ranges of all call bout and call variables of this and the previous sample overlap broadly. The populations are therefore considered to be conspecific.

3.5.4 Loc. 55 (Wakkerstroom) and Loc. 43 (Piet Retief).

These two localities are 60km apart and are situated in similar habitats. Locality 55 is the farm on which adult specimens and immature stages were collected and described in 1929 by Fitzsimons & van Dam (as *B. parvus*), and in 1970 by Swanepoel (as *B.a. pentheri*).

The raw data for the three specimens is presented in Table 27. The call variables all fall within the ranges of the Belfast-Lydenburg sample (Table 25, Fig.16), with the exception of the specimen from Wakkerstroom, which differs markedly in call duration and pulse number. Calls of the latter were recorded at a temperature well below the minimum temperature of the Belfast-Lydenburg sample. Thus adjustment of these variables to the mean of the Belfast-Lydenburg sample would probably bring them within the range of that population. These three populations are therefore considered to be conspecific with the previous two samples.

3.5.5 Loc. 8 (Graskop 1).

This fairly large sample from a single locality (Table 28, Fig.16 & 17) is unusual in that the minimum and median body mass is substantially lower than that of any of the previous samples. However there is an overlap in the ranges in body mass, and the difference is therefore attributed to geographical variation.

In comparison with the Belfast-Lydenburg sample (Table 25), dominant frequency in this sample extends much higher (to 2927Hz). This difference is probably due to the lower minimum body mass of 0.67g in the Graskop sample cf. 1.21g in the other sample.

The temperature ranges of the samples overlap broadly and a direct comparison of the temporal variables is thus possible. There is a broad overlap in the ranges of all temporal call bout and call variables. The two samples are therefore considered to be conspecific.

3.5.6 Loc. 9 (Graskop 2), Loc. 44 (Pilgrim's Rest) & Loc. 55 (Trichardtsdal).

These localities represent additional distribution records for the species. Call recordings and specimens were collected (by Dr R. Toms at the first two localities). The advertisement calls are similar in structure to the Graskop sample (the data are not presented in this thesis).

TABLE 25. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.2 (Belfast) and Loc.27 (Lydenburg 1).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	14	2329	201	105	1950	2623	2313	267	0.05	8.64
Call duration (s)	14	0.091	0.034	0.018	0.054	0.173	0.079	0.030	2.26	37.37
Number of pulses	14	7	1.5	0.8	6	10	8	3	0.58	18.93
Pulse rate (s^{-1})	14	88	20.4	10.7	55	118	87	37	-0.16	23.23
% grouped calls	14	22	21	11	0	64	16	36	1.21	97.44
Call int. in group (s)	9	0.798	0.144	0.094	0.560	1.032	0.809	0.127	0.12	18.07
Air temperature (°C)	14	13.1	2.3	1.2	10.2	17.6	12.7	3.9	1.18	17.67
Body mass (g)	14	2.19	0.95	0.50	1.21	4.96	2.11	1.06	2.97	43.52

TABLE 26. Summary statistics of advertisement call variables, air temperature and body mass of populations of *B. mossambicus* sampled at Loc.31, 32 & 33 (Mbabane 1, 2, & 3).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	10	2587	102	63	2390	2730	2573	122	-0.65	3.92
Call duration (s)	10	0.057	0.010	0.006	0.044	0.075	0.053	0.016	0.96	17.79
Number of pulses	10	8	1.2	0.7	6	10	8	1	0.94	15.06
Pulse rate (s^{-1})	10	125	16.1	9.9	106	160	125	20	1.25	12.91
% grouped calls	10	15	21	13	0	48	0	38	1.22	142.95
Call int. in group (s)	4	0.528	0.099	0.097	0.409	0.648	0.527	0.143	0.05	18.85
Air temperature (°C)	10	16.2	2.3	1.43	14.0	21.0	15.5	3.6	1.32	14.27
Body mass (g)	7	1.92	0.48	0.36	1.36	2.70	1.75	0.84	0.72	25.01

TABLE 27. Advertisement call variables, air temperature and body mass of three individuals of *B. mossambicus* from Loc.56 (Wakkerstroom) and Loc.43 (Piet Retief).

Variable	Locality 55	Locality 56	
	No. 300	No.301	No. 303
Raw data:			
Dom. frequency (Hz)	2037	2157	2287
Call duration (s)	0.258	0.127	0.108
Number of pulses	10	10	8
Pulse rate (s^{-1})	38	77	67
Air temperature (°C)	7.4	12.3	11.9
Body mass (g)	1.94	--	1.68

TABLE 28. Summary statistics of advertisement call variables, air temperature and body mass of a population of *B. mossambicus* sampled at Loc.8 (Graskop 1).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	22	2710	117	49	2513	2927	2719	190	-0.23	4.31
Call duration (s)	22	0.073	0.014	0.006	0.044	0.104	0.073	0.018	0.48	18.98
Number of pulses	22	7	1.5	0.6	4	9	7	2	-0.47	22.34
Pulse rate (s^{-1})	22	89	21.4	8.9	57	134	93	29	0.83	23.96
% grouped calls	22	22	24	10	0	78	13	42	1.88	111.23
Call int. in group (s)	11	0.694	0.212	0.125	0.425	1.071	0.660	0.320	0.77	30.54
Air temperature (°C)	22	15.5	3.9	1.6	10.5	26.0	15.2	2.9	2.23	25.18
Body mass (g)	18	1.09	0.30	0.14	0.67	1.67	0.99	0.48	0.76	27.31

3.6 Comparison of the coastal and escarpment samples of *B. mossambicus*.

The advertisement call data of the populations sampled along the eastern escarpment (3.5.2 - 3.5.5) are combined in Table 29 for comparison with the coastal populations sampled in southern Mozambique and KwaZulu/Natal (Table 24).

A direct comparison of dominant frequency is possible because two samples have similar medians and ranges in body mass. The median dominant frequency differs by only 137Hz in the two samples and the ranges of this variable are congruent.

The median air temperatures of the two samples differ by 5.7°C, but their ranges overlap quite broadly. The data do not yield strong correlation coefficients for all call variables: consequently these variables cannot be adjusted. Instead, a portion of the data which lies between a common, narrow temperature range is selected from each sample for the purpose of comparison (Table 30). This comparison reveals a very close similarity between the samples in respect of all call variables. The populations from KwaZulu/Natal and those from the escarpment, previously referred to *B.a. pentheri*, are therefore considered to be conspecific and are referred to *B. mossambicus* (see 3.8). These data are combined in Table 31.

TABLE 29. Summary statistics of advertisement call variables, air temperature and body mass of combined samples of *B. mossambicus* from the Escarpment.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	52	2505	273	74	1867	2927	2587	398	-2.32	10.91
Call duration (s)	52	0.087	0.047	0.013	0.044	0.258	0.074	0.031	6.92	53.61
Number of pulses	52	7	1.6	0.4	4	11	7	2	0.07	21.39
Pulse rate (s^{-1})	52	91	27.9	7.6	38	160	93	42	0.17	30.56
% grouped calls	52	18	22	6	0	78	10	36	2.89	118.28
Call int. in group (s)	24	0.705	0.192	0.008	0.409	1.071	0.683	0.270	0.61	27.28
Air temperature (°C)	52	14.3	3.8	1.0	7.4	26.0	14.1	4.7	2.01	26.29
Body mass (g)	42	1.81	1.01	0.31	0.67	4.96	1.50	1.26	4.05	55.84

TABLE 30. Summary statistics of call data from coastal KwaZulu/Natal and the escarpment which lie within the air temperature range: 16.1°C to 18.8°C.

Variable	N	Mean	Min	Max
Coastal KwaZulu/Natal				
Temperature (°C)	10	17.3	16.1	18.8
Call duration (s)	10	0.069	0.044	0.104
Number of pulses	10	7.5	6	10
Pulse rate (s^{-1})	10	104	74	131
Escarpment				
Temperature (°C)	10	17.6	16.6	18.3
Call duration (s)	10	0.083	0.050	0.101
Number of pulses	10	9.6	7	12
Pulse rate (s^{-1})	10	105	88	119

TABLE 31. Summary statistics of advertisement call variables, air temperature and body mass of all populations from Zululand and the Escarpment assigned to *B. mossambicus*, and the same data adjusted to the median mass and air temperature of the combined sample from northern Mozambique (Table 16).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	123	2460	215	38	1863	2927	2498	240	-2.90	8.74
Call duration (s)	123	0.082	0.032	0.006	0.044	0.258	0.077	0.022	14.9	39.21
Number of pulses	123	10	2.7	0.5	4	18	9	3	2.07	28.03
Pulse rate (s^{-1})	123	115	33	11.5	38	202	113	40	-0.50	28.71
% grouped calls	107	27	30	6	0	100	15	49	3.02	109.26
Call int. in group (s)	66	0.507	0.202	0.049	0.240	1.071	0.44	0.252	3.72	39.83
Air temperature (°C)	122	17.3	4.3	0.8	7.4	29.5	17.7	5.4	0.52	24.58
Body mass (g)	100	2.39	1.36	0.27	0.67	6.27	1.9	1.2	4.65	56.87
Data adjusted to 8.14g and 26.4°C:										
Dom. frequency (Hz)	99	1856			1259	2324	1894			
Call duration (s)	121	0.052			0.014	0.228	0.047			
Pulse rate (s^{-1})	121	165			88	252	163			

3.7 Differences between the southern populations assigned to *B. mossambicus* and the populations assigned to *B.a.adspersus*.

In 3.2.4 the advertisement calls of the northern Mozambique populations of *B. mossambicus* was compared with the Pietersburg population of *B.a. adspersus*, and found to differ in several respects, viz. call duration, number of pulses and the percentage of grouped calls in the call bout. The comparison involved two allopatric populations separated by a very large distance.

This is not the case with the present comparison which involves populations situated much closer together, sometimes calling within earshot of one another. In such instances, population-based call differences are much more obvious and more significant in that they clearly indicate the existence of separate gene pools.

In the interior, the southern populations referred to *B. mossambicus* appear to be separated from *B.a. adspersus* by different habitat preferences: the former occur at higher altitudes in montane grassland while the latter occupy the drier savannah away from the mountains. On the coastal plain, differences in habitat preference are not apparent: populations referred to *B.a. adspersus* were found at relatively few localities (16, 49 & 50), whereas those referred to *B. mossambicus* were more commonly encountered. The two species occur in parapatry at Lydenburg 2 (Loc. 28), Trichardtsdal (Loc.55), between Mbabane 2 (Loc.32) and Manzini, and between Sihangwana 3 & 4 (Loc.48&49).

The advertisement call data of the southern *mossambicus* populations (Table 31) are compared below with the combined data from a number of *adspersus* populations, viz: Loc.42 (Pietersburg), Loc.14 (Houtbosdorp 1), Loc.28 (Lydenburg 2), Loc.57 (White River), Loc.34 (Mica), Loc.13 (Hoedspruit), Loc.21 (Komatipoort 1), Loc.3 (Burchenough Bridge) and Loc.38 (Mutare 1), in Table 32..

The samples of the two species differ markedly in body mass. When the *adspersus* data is corrected to the mean body mass of the *mossambicus* sample (1.9g), the mean

dominant frequency of the *B.a. adspersus* sample rises to 2181Hz (range = 1580 - 2969Hz; median = 2167Hz), 279Hz below the mean of the *B. mossambicus* sample. A difference of this magnitude was not observed in the comparison between *adspersus* from Pietersburg and the northern *mossambicus* (3.2.4).

Because the means and ranges in air temperature of the two samples are similar, the temporal call variables of the *adspersus* and *mossambicus* samples may be compared directly. As was the case in the comparison between *adspersus* and northern *mossambicus*, significant differences involve call duration, number of pulses and the percentage of grouped calls in the call bout, while pulse rate and the call interval within groups are very similar.

Thus the southern populations assigned to *mossambicus* differ from *adspersus* in much the same way as do the northern populations, and the nature and extent of the differences indicate that two separate genetical species are represented.

TABLE 32. Summary statistics of advertisement call variables, air temperature and body mass of all populations referred to *B.a. adspersus* .

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Raw data:										
Dom. frequency (Hz)	122	1743	100	18	1482	2197	1733	136	3.11	5.73
Call duration (s)	122	0.193	0.047	0.008	0.074	0.293	0.192	0.061	-0.72	24.45
Number of pulses	122	23	3.3	0.6	14	31	23	4	-1.73	14.52
Pulse rate (s^{-})	122	121	27.1	4.8	71	226	120	35	4.82	22.41
% grouped calls	120	77	25	4	0	100	84	23	-7.28	32.28
Call int. in group (s)	115	0.512	0.125	0.23	0.273	0.820	0.485	0.179	1.80	24.40
Air temperature (°C)	122	16.3	3.1	0.5	9.5	23.1	16.4	4.9	-0.04	18.89
Body mass (g)	88	6.43	1.47	0.31	2.91	9.87	6.38	2.14	0.09	22.83

3.8 Comparison of the southern and northern samples of *B. mossambicus*.

Advertisement call data indicate that the Island and mainland populations of *B. mossambicus* in northern Mozambique are conspecific (3.3.1), in spite of differences in size and dorsal colouring (Fig.3). Similarly, it has been demonstrated that in the south, populations which occupy the coastal plain and the eastern escarpment, and exhibit a wide variation in colouration and markings (Fig.17), are also conspecific (3.6).

It is now necessary to examine the relationship between the northern Mozambique and the southern populations in the light of the available evidence.

Morphologically, the southern populations differ from the northern populations in possessing light dorsal and dorsolateral markings. Similar markings are present in *B. adspersus* (Fig.2) and have been used to distinguish this species from the northern Mozambique populations of *B. mossambicus* (3.1). It could therefore be argued, on the basis of morphological evidence alone, that the southern populations are not related to *B. mossambicus* but rather to *B.a. adspersus*.

However,once the southern sample has been adjusted to the median body mass and air temperature of the northern sample, a comparison of the advertisement calls of the two samples (Tables 16 & 31) reveals no substantial differences: mean dominant frequency ($r = -0.61$, $m = -96.88$) differs by only 21Hz. Mean call duration ($r = -0.46$, $m = -.0035$) differs by 0.002s, and the mean pulse rate ($r = 0.74$, $m = 5.75$) differs by $22s^{-1}$. The mean number of pulses differs by only one pulse.

Therefore, since no differences are apparent in the advertisement calls (SMRS) of the two samples, the northern and southern populations are considered to be conspecific. Consequently, light dorsal markings can no longer be used to distinguish *B. mossambicus* from *B. adspersus*, and other morphological characters will have to be sought.

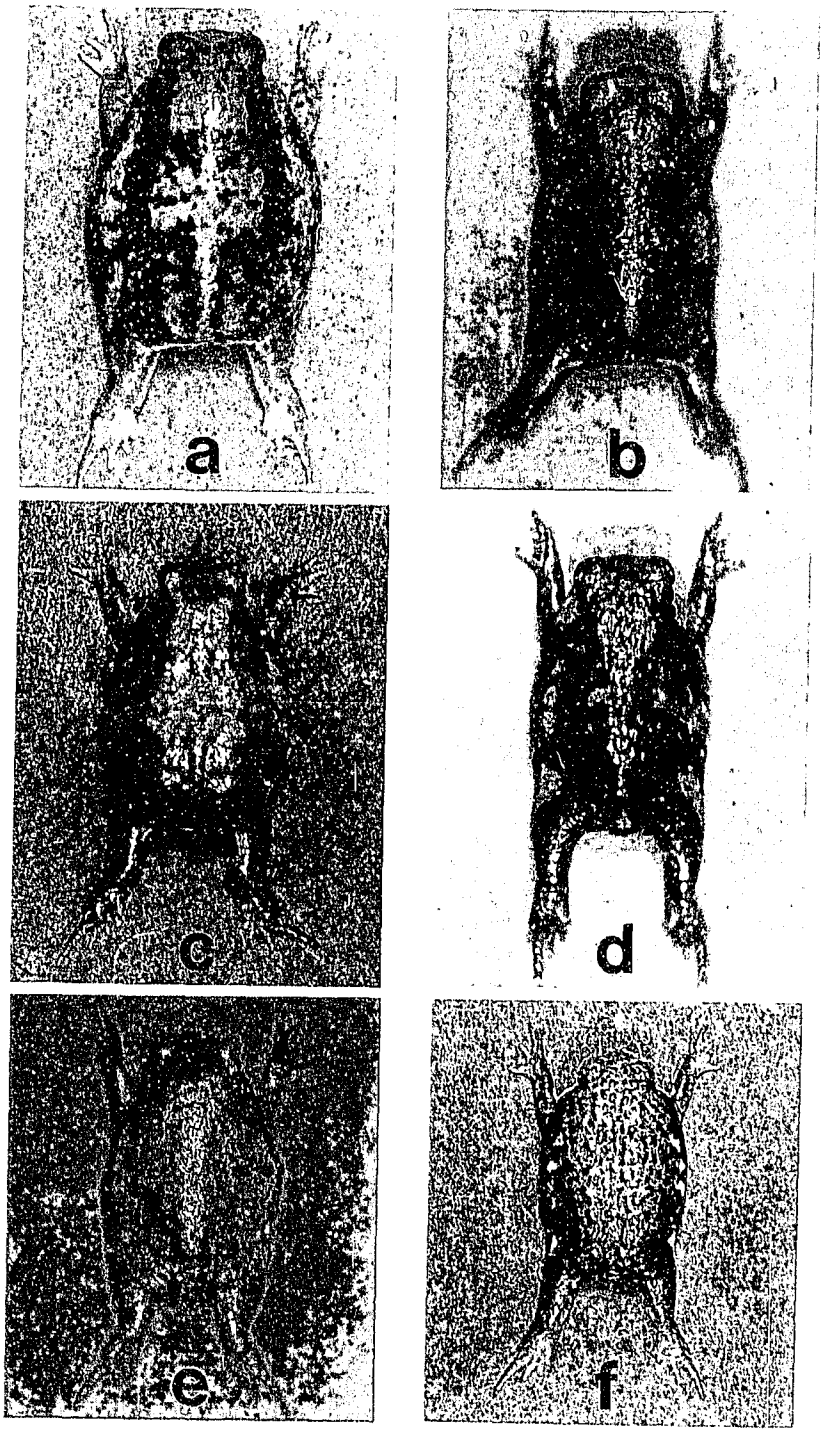

FIGURE 17. Dorsal colouration and markings of *B. mossambicus* males from: (a) Loc.48 (Sihangwane 3); (b) Loc.51 (St Lucia 1); (c) Loc.37 (Mtunzini); (d) Loc.6 (Eshowe); (e) Loc.27 (Lydenburg 1); (f) Loc.8 (Graskop 1).

4. Group 3 (*Breviceps verrucosus* Rapp 1842)

4.1 Introduction.

The calls of 34 males were collected at six localities in the Eastern Cape Province, KwaZulu/Natal and Mpumalanga. Both subspecies, *verrucosus* and *tympanifer* are represented. The dorsal colouring and markings closely match the substrate: those occurring on dark loamy soils or the forest floor tend to be uniformly dark brown or black (Fig.4 a&b), while those lighter sandy or clay soils are light brown with a varying amount of dark brown mottling (Fig.4c&d).

4.2 Populations sampled.

4.2.1 Locality 54 (Stutterheim)

This sample comprises eight specimens of *B.v. tympanifer* collected in open grassland (Fig.4c&d). The calls are pulsatile and are not grouped, but repeated continuously for up to 46 seconds at a mean call rate of 26 . ·lls per minute (Table 33, Fig.19). Frequency modulation occurs in a few calls, with the frequency rising towards the end of the call. Sidebands are present. Individual calls are about 0.53s in duration and consist of about 72 pulses emitted at a pulse rate of 136 pulses per second. Call parameters have a low coefficient of variation but bout parameters are more variable (Table 33).

4.2.2 Locality 12 (Harding)

Ten specimens were collected from closed canopy forest and from the side of a road passing through an indigenous forest. Eight specimens are very dark, resembling "typical" *B.v. verrucosus*, but two specimens exhibit lighter "*tympanifer*" markings.

The data could not be corrected for mass and temperature because of the small sample size (Table 34). However, the call bout and call variables are directly comparable with the Stutterheim sample because the medians for body mass and air temperature

are very similar, although the differences in their ranges should also be noted. The two samples are very similar with regard to call bout variables, and the ranges in the dominant frequency and pulse rate of the call overlap broadly. The ranges of call duration and number of pulses in the two samples differ slightly, but are not widely separated. Because of the close similarities in the advertisement calls, the populations are considered to be conspecific.

4.2.3 Locality 45 (Seymour)

The calls of two specimens of *B.v. tympanifer* were collected in montane grassland, during daylight: only a few widely scattered individuals were calling. The considerable difference between air and cloacal temperature in specimen No. 410 was due to the low temperature of the clay soil, and the insulating effect of the grass cover. The bout and call variables of this sample (Table 35) fall within the ranges of the previous two samples and they are therefore regarded as conspecific.

4.2.4 Locality 6 (Eshowe)

These specimens were collected in a suburban garden: their natural habitat is not known, as both grassland and forest occur in the area. They are almost completely light brown above with a few darker speckles or spots, presenting a pattern intermediate between *verrucosus* and *tympanifer*.

The calls of these four specimens (Table 36) can be compared directly with the Stutterheim and Harding samples because of the similar medians and ranges in body mass and air temperature. Only a few individuals were calling intermittently at this locality, which probably accounts for the low call rate. The medians and ranges of other call bout variables are similar to those of the other samples. The call variables of the samples are also closely matched, with the exception of dominant frequency which, in the Eshowe sample, overlaps in the lower part of its range but reaches a maximum about 200Hz higher than the other samples. These specimens are also considered to be conspecific with the previous samples.

4.2.5 Locality 27 (Lydenburg 1)

The seven specimens forming this sample were collected in montane grassland, where they formed part of a moderately strong chorus. They exhibit the *tympanifer* markings on the dorsal surface.

The medians and means of all call bout and call variables of this sample (Table 37) correspond with or broadly overlap those of the previous samples. The population is therefore also assigned to *B. verrucosus*.

4.2.6 Locality 8 (Graskop 1)

This sample comprises three specimens collected from beneath leaf litter on the fringes of indigenous mist-forest. They are uniform black above with scattered small yellow speckles, conforming to the *verrucosus* pattern (Fig.4a&b).

The call variable ranges of this sample (Table 38; Fig.18) cannot be distinguished from the other samples of this species. Specimen No. 131 has a longer call duration than was recorded in any of the other samples, but the other two specimens fall well within the "normal" range.

4.3 Advertisement call summary statistics for *B. verrucosus*.

The data show that there are no grounds for the recognition of separate species or subspecies among the sampled populations, nor can the criterion of geographical isolation be invoked to justify the recognition of subspecies. The variation in dorsal pattern seems rather to be an adaptation to the local substrate, ie. crypsis.

The data are therefore combined in Table 39 to generate summary statistics for the entire sample. Of all the call variables, dominant frequency has the lowest coefficient of variation, followed by pulse rate and call duration.

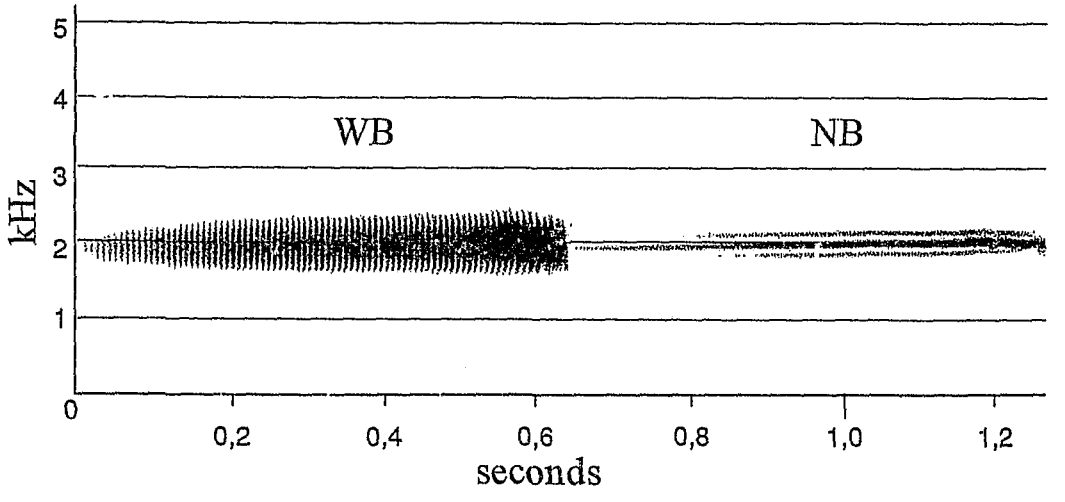

FIGURE 18. Wide (WB) and narrow band (NB) sonagrams of the advertisement call of *B. verrucosus verrucosus* from Loc.8 (Graskop 1).

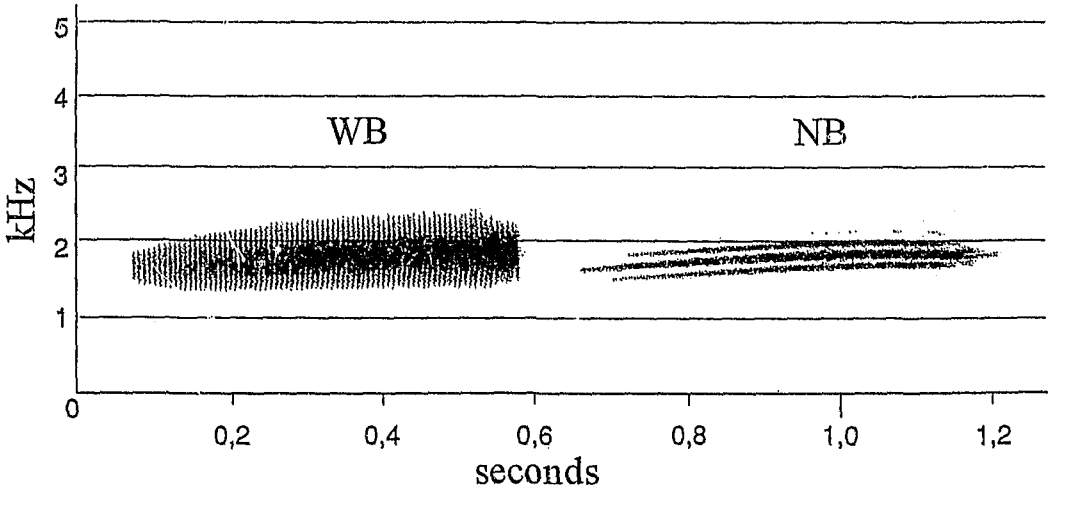

FIGURE 19. Wide (WB) and narrow band (NB) sonagrams of the advertisement call of *B. verrucosus tympanifer* from Loc.54 (Stutterheim).

TABLE 33. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. verrucosus* sampled at Loc.54 (Stutterheim).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	7	7.42	1.34	1.00	5.35	8.68	7.68	2.92	-0.87	18.11
Air temperature (°C)	8	14.7	0.2	0.2	14.3	15	14.8	0.3	-0.66	1.48
Bout variables:										
Bout duration (s)	8	29	11	8	12	46	30	16	-0.05	38.44
Number of calls	8	12	4.9	3.4	6	19	10	8.5	0.59	42.60
Call rate (min^{-1})	8	26	5.5	3.7	16	33	26	7	-0.78	21.11
Call interval (s)	8	2.097	0.365	0.414	1.770	2.490	2.030	0.720	0.56	17.39
Call variables:										
Dom. frequency (Hz)	8	1907	81	56	1753	2033	1912	69	-0.70	4.25
Call duration (s)	8	0.529	0.035	0.024	0.474	0.579	0.517	0.047	0.19	6.61
Number of pulses	8	72	2.9	2.0	67	76	72	3	-0.06	3.98
Pulse rate (s^{-1})	8	136	9.4	6.5	125	149	135	17	0.18	6.93

TABLE 34. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. verrucosus* sampled at Loc.12 (Harding).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	8	8.97	2.97	2.00	5.00	13.59	8.10	4.65	0.41	33.16
Air temperature (°C)	10	16.0	2.5	1.56	13.8	20.8	15.3	1.9	1.77	15.66
Bout variables:										
Bout duration (s)	10	23.4	7.5	4.6	13	36	23	11	0.50	32.06
Number of calls	10	10	3.4	1.5	5	16	9	4	0.78	35.52
Call rate (min^{-1})	10	24	1.8	1.1	21	27	24	2	-0.01	7.53
Call interval (s)	4	2.205	0.241	0.237	1.900	2.490	2.215	0.300	-0.20	10.94
Call variables:										
Dom. frequency (Hz)	10	1720	144	89	1550	2005	1710	219	0.87	8.38
Call duration (s)	10	0.700	0.052	0.033	0.625	0.793	0.696	0.076	0.59	7.48
Number of pulses	10	102	19	12	75	139	101	16	0.77	18.74
Pulse rate (s^{-1})	10	145	22	14	114	188	141	25	0.72	14.98

TABLE 35. Call bout and advertisement call variables, air temperature and body mass of two specimens of *B. verrucosus* sampled at Loc.45 (Seymour).

Variables	No. 305	No. 410
Body mass (g)	13.4	15.5
Air temperature (°C)	7.4	23.3 (cloacal = 17.0)
Bout variables:		
Bout duration (s)	65	23
Number of calls	17	6
Call rate (min^{-1})	16	16
Call variables:		
Dom. frequency (Hz)	1642	1612
Call duration (s)	0.597	0.494
Number of pulses	52	64
Pulse rate (s^{-1})	87	128

TABLE 36. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. verrucosus* sampled at Loc.6 (Eshowe).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	3	8.71	2.07	2.33	6.55	10.67	8.92	4.12	-0.31	23.73
Air temperature (°C)	4	15.5	0.8	0.8	14.6	16.2	15.5	1.4	-0.08	5.30
Bout variables:										
Bout duration (s)	3	32	14.7	16.7	23	49	24	26	1.22	46.03
Number of calls	3	7	3.1	3.5	4	10	6	6	0.66	45.83
Call rate (min^{-1})	3	13	2.1	2.4	11	15	12	4	0.92	16.43
Call variables:										
Dom. frequency (Hz)	4	2092	123	121	1987	2238	2072	204	0.34	5.89
Call duration (s)	4	0.625	0.053	0.052	0.561	0.674	0.633	0.088	-0.37	8.54
Number of pulses	4	93	8	8	82	99	96	11	-1.29	8.37
Pulse rate (s^{-1})	4	151	5	5	146	157	150	9	0.24	3.59

TABLE 37. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. verrucosus* sampled at Loc.27 (Lydenburg 1).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	5	11.22	6.36	5.57	7.52	22.53	8.58	1.18	1.99	56.67
Air temperature (°C)	7	13.3	0.6	0.4	12.6	14.3	13.2	1	0.88	4.52
Bout variables:										
Bout duration (s)	7	37	12.0	8.9	20	56	35	20	0.45	32.55
Number of calls	7	12	3.0	2.5	7	16	11	7	0.40	29.03
Call rate (min^{-1})	7	19	1.2	0.9	17	21	19	1	-0.40	6.35
Call variables:										
Dom. frequency (Hz)	7	1771	51	38	1688	1826	1777	88	-0.69	2.89
Call duration (s)	7	0.543	0.089	0.066	0.421	0.696	0.515	0.118	0.76	16.45
Number of pulses	7	69	11	8	53	87	68	14	0.41	15.59
Pulse rate (s^{-})	7	128	2.4	1.8	124	131	129	4	0.08	1.91

TABLE 38. Call bout and advertisement call variables, air temperature and body mass of three specimens of *B. verrucosus* sampled at Loc.8 (Graskop 1).

Variables	No. 130	No. 131	No. 132
Body mass (g)	4.47	9.37	4.44
Air temperature (°C)	13.2	15.5	11.4
Bout variables:			
Bout duration (s)	18	26	20
Number of calls	6	8	6
Call rate (min^{-1})	20	19	18
Call variables:			
Dom. frequency (Hz)	1973	1797	1978
Call duration (s)	0.675	0.912	0.656
Number of pulses	89	123	90
Pulse rate (s^{-})	131	136	138

TABLE 39. Summary statistics of call bout and advertisement call variables, air temperature and body mass of the combined samples of *B. verrucosus*.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	28	9.04	3.79	1.40	4.44	22.53	8.49	3.11	4.02	41.93
Air temperature (°C)	34	14.8	2.7	0.9	7.4	23.3	14.7	1.6	2.07	18.01
Bout variables:										
Bout duration (s)	33	29.5	12.5	4.3	12	65	26	15	2.51	42.41
Number of calls	33	10	4.1	1.4	4	19	9	4	1.55	40.54
Call rate (min^{-1})	33	22	5.1	1.8	11	33	21	6	0.20	23.81
Call interval (s)	7	2.159	0.277	0.206	1.770	2.490	2.210	0.590	-0.06	12.83
Call variables:										
Dom. frequency (Hz)	34	1830	164	55	1550	2238	1813	238	0.79	8.96
Call duration (s)	34	0.612	0.105	0.035	0.421	0.912	0.612	0.159	1.49	17.12
Number of pulses	34	84	10.2	6.8	52	139	79	29	1.91	24.00
Pulse rate (s^{-})	34	137	17.1	5.8	87	188	137	19	0.57	12.51

5. Group 4 (*Breviceps sylvestris* FitzSimons 1930)

5.1 Introduction.

The advertisement calls of 23 males of both subspecies, *sylvestris* and *taeniatus* were collected from two localities in the Northern Transvaal. Like *B. verrucosus*, they occur in forest and grassland habitats and exhibit a wide range of colouration and markings. Some specimens are entirely black, dorsally, while others are a uniform light brown. The diagnostic characters of the subspecies described by Poynton (1964) are not consistently exhibited by all individuals (see below).

5.2 Populations sampled.

5.2.1 Locality 15 (Houtbosdorp 2)

The 13 specimens in this sample were collected from closed canopy indigenous forest, as well as from open grassland 300m from the forest margin (Fig.5a&b). They are variable in colouration and markings, ranging from uniform light brown to black, or mottled. Some specimens possess indistinct, oblique transverse bars on the dorsal surface: this has been used as a diagnostic character for *B.s. taeniatus* (Poynton, 1964). The specimens are assigned to *B.s. sylvestris* as they occur within its known range.

The calls are pulsatile and are not grouped. They are repeated continuously for up to 28 seconds at a mean call rate of 68 calls per minute (Table 40, Fig.20). A few calls show slight frequency modulation, with the frequency rising towards the end of the call. Sidebands are present in the narrow band frequency spectrum (Fig.20, NB). Call bout characters are highly variable, while call characters are less variable: dominant frequency has the lowest coefficient of variation, followed by number of pulses, and call duration.

5.2.2 Locality24 (Louis Trichardt)

This sample of 10 specimens was collected in indigenous forest and fringing grassland in the Soutpansberg, . The transverse bands characteristic of *B.s. taeniatus* are present in most (not all) specimens to a varying degree. However in most specimens, the dorsal glandular ridges converge in the scapular region, a character regarded by Poynton (1964) as diagnostic for *B.s. sylvestris*. The specimens are assigned to *B.s. taeniatus* as they occur within the known range of this subspecies.

The advertisement call data are presented in Table 41 & Fig.21. The comparison of the two samples of this species is complicated by the fact that their ranges in body mass and air temperature do not correspond. Correction factors could not be calculated because of the small sample size. However, most bout and call variables overlap, and correction of the data would tend to increase the degree of correspondence between them. The only call variable which does not overlap is dominant frequency, but the distance between the ranges is small and the difference would be reduced by correction of the data to a common mass. The two samples are therefore regarded as being conspecific and the data are combined in Table 42.

5.3 Comparison of the calls of *B. verrucosus* and *B. sylvestris*.

The samples of these two species are fairly similar in respect of median body mass and air temperature and may therefore be compared directly. With regard to call bout variables, the two species differ in the call rate and the interval between calls, although the ranges of these characters are not widely separated.

The means and ranges of dominant frequency and pulse rate correspond well, while the ranges of call duration and number of pulses overlap only slightly. The only differences between the species are that the calls of *B. verrucosus* are usually longer and produced at a slower rate than those of *B. sylvestris*. Whether these differences are of sufficient magnitude to constitute a species specific mate recognition character is debatable. It is thus possible that *B. sylvestris* and *B. verrucosus* are conspecific.

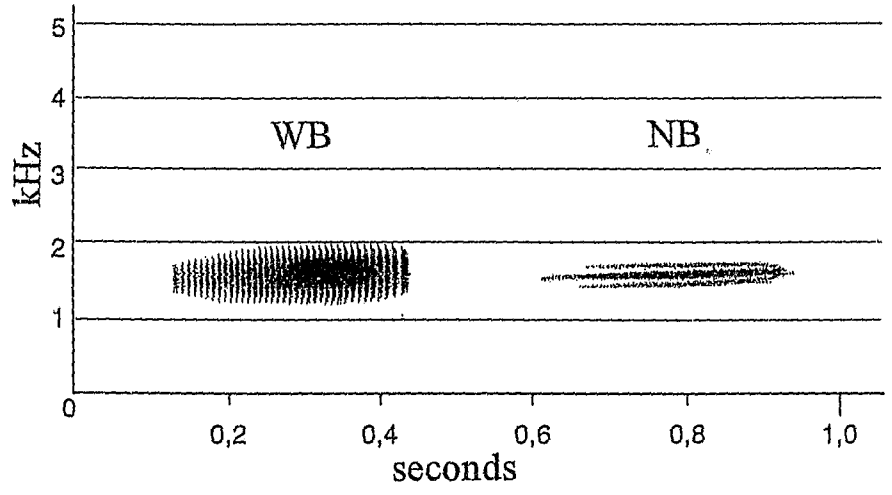

FIGURE 20. Wide (WB) and narrow band (NB) sonagrams of the advertisement call of *B. sylvestris sylvestris* from Loc.15 (Houtbosdorp 2).

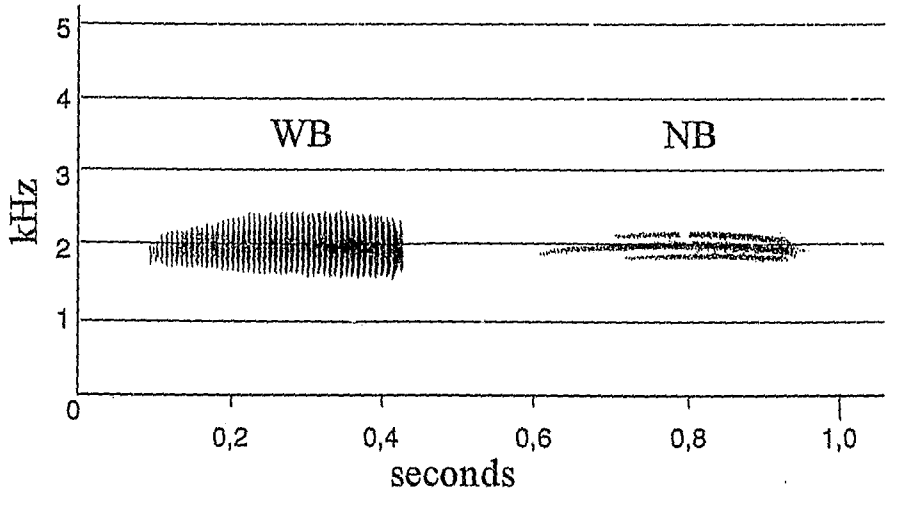

FIGURE 21. Wide (WB) and narrow band (NB) sonagrams of the advertisement call of *B. sylvestris taeniatus* from Loc.24 (Louis Trichardt).

TABLE 40. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. sylvestris sylvestris* sampled at Loc.15 (Houtbosdorp 2).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	13	8.61	1.39	0.76	6.39	10.49	8.60	1.76	-2.27	16.11
Air temperature (°C)	13	10.9	2.2	1.2	8.8	15.2	9.9	2.1	1.37	19.20
Bout variables:										
Bout duration (s)	13	14	6.1	3.3	8	28	12	5	2.31	44.26
Number of calls	13	14	4.1	2.3	10	25	13	4	2.46	29.25
Call rate (min^{-1})	13	68	20.4	11.1	45	115	62	19	1.81	30.20
Call interval (s)	13	0.911	0.197	0.108	0.625	1.238	0.946	0.214	0.16	21.59
Call variables:										
Dom. frequency (Hz)	13	1599	76.9	41.8	1496	1775	1586	74	1.45	4.81
Call duration (s)	13	0.395	0.071	0.039	0.282	0.504	0.406	0.118	-0.25	18.06
Number of pulses	13	37	3.1	1.7	30	42	37	3	-0.74	8.33
Pulse rate (s^{-1})	13	92	18.9	10.3	73	132	84	12	1.74	20.59

TABLE 41. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. sylvestris taeniatus* sampled at Loc.24 (Louis Trichardt 1).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	10	5.45	0.73	0.45	4.29	6.20	5.69	1.46	-0.89	13.32
Air temperature (°C)	10	15.9	1.0	0.6	14.4	17.7	15.9	1.1	0.22	6.19
Bout variables:										
Bout duration (s)	9	8	4.0	2.6	3	17	7	1	1.79	50.88
Number of calls	9	9	3.7	2.4	4	16	9	5	0.70	41 50
Call rate (min^{-1})	9	72	10.4	6.8	53	87	73	9	-0.58	14.39
Call interval (s)	10	0.830	0.157	0.097	0.588	1.082	0.794	0.167	0.67	18.95
Call variables:										
Dom. frequency (Hz)	10	1926	82	51	1825	2057	1921	118	0.29	4.26
Call duration (s)	10	0.316	0.037	0.024	0.262	0.404	0.314	0.024	1.61	11.79
Number of pulses	10	44	4.3	3	38	53	43	6	1.45	9.83
Pulse rate (s^{-})	10	138	12.2	7.6	120	162	136	10	0.87	8.86

TABLE 42. Summary statistics of call bout and advertisement call variables, air temperature and body mass of all samples of of *B. sylvestris* combined.

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	23	7.24	1.96	0.79	4.29	10.49	6.86	2.81	0.58	27.08
Air temperature (°C)	23	13.1	3.0	1.2	8.8	17.7	14.1	6	-0.38	23.06
Bout variables:										
Bout duration (s)	22	11.3	5.9	2.5	3	28	10	5	2.71	52.76
Number of calls	22	12	4.7	1.9	4	25	11	4	1.51	39.09
Call rate (min^{-1})	22	69	16.8	7.1	45	115	67	18	1.68	24.28
Call interval (s)	23	0.876	0.182	0.074	0.588	1.238	0.870	0.276	0.67	20.71
Call variables:										
Dom. frequency (Hz)	23	1741	183	75	1496	2057	1701	323	0.55	10.50
Call duration (s)	23	0.361	0.070	0.029	0.262	0.504	0.328	0.116	1.15	19.51
Number of pulses	23	40	4.9	2.0	30	53	39	5	1.30	12.63
Pulse rate (s^{-})	23	112	28.2	11.5	73	162	120	52	0.03	25.12

6. Group 5 (*Breviceps poweri* Parker 1934)

6.1 Introduction.

A single sample of 40 specimens was collected at Loc.40 (Nampula, northern Mozambique) in a cashew nut plantation, following heavy rain (Fig.6a&b). *B. mossambicus* was also breeding in large numbers at the same locality. The chorus began in the early evening and reached its peak intensity soon after dark, continuing through the night until dawn.

The specimens exhibit diagnostic characters indicated by Poynton & Broadley (1985) as diagnostic of *B. poweri*, viz:a light urostylar spot and a series of 3 - 6 light dorsolateral patches. The advertisement calls are similar to those recorded at Solwezi, Zambia, by Prof. A. Channing (sonagram examined). The latter locality is 350km NW of the type locality (Kabwe, Zambia) of *B. poweri*, and 1450km WNW of Nampula.

6.2 Advertisement call structure.

6.2.1 Structure and variation of call bouts.

In a sample of 47 call bouts from 40 males, the bout lasted for up to 30 seconds and consisted of 10 - 74 calls emitted at a mean rate of 175 call per minute (Table 43). Most (99.6%) of the calls were grouped : 43% formed groups of 4 - 5 calls, while 39% formed larger groups of up to 17 calls (Figs.22 & 27c). The interval between two calls within a call group was considerably shorter than the interval between two call groups. In contrast to *B.a. adspersus*, the call groups are larger and the calls are closer together within the group.

6.2.2 Structure and variation of individual calls.

The call is a short, unpulsed whistle with a slow rise time and a rapid fall time (Fig. 23). The oscillogram reveals the presence of masked amplitude modulation in the call

(Fig.24). It is interesting that there are as many "hidden pulses" in the call of *B. poweri* as there are pulses in the call of *B. adspersus*. However, the "hidden pulses" do not generate sidebands in the narrow band sonagram (Fig. 23, NB), and it is unlikely that they function in mate recognition. An harmonic series is present, and the fundamental frequency is dominant. Frequency modulation occurs in the form of a slight rise in frequency towards the end of the call.

Dominant frequency, call duration and call interval within groups all exhibit a low coefficient of variation, ie. they are highly stable call characters. Because of the narrow temperature range over which the calls were recorded, the data do not reflect significant correlations between call variables and air temperature; the correlation between body mass and dominant frequency was relatively low ($r=-0.52$, $p=.001$, $N=39$).

The advertisement call in this sample of *B. poweri* resembles that of *B.a. adspersus* in duration and dominant frequency, but differs in being essentially non-pulsatile.

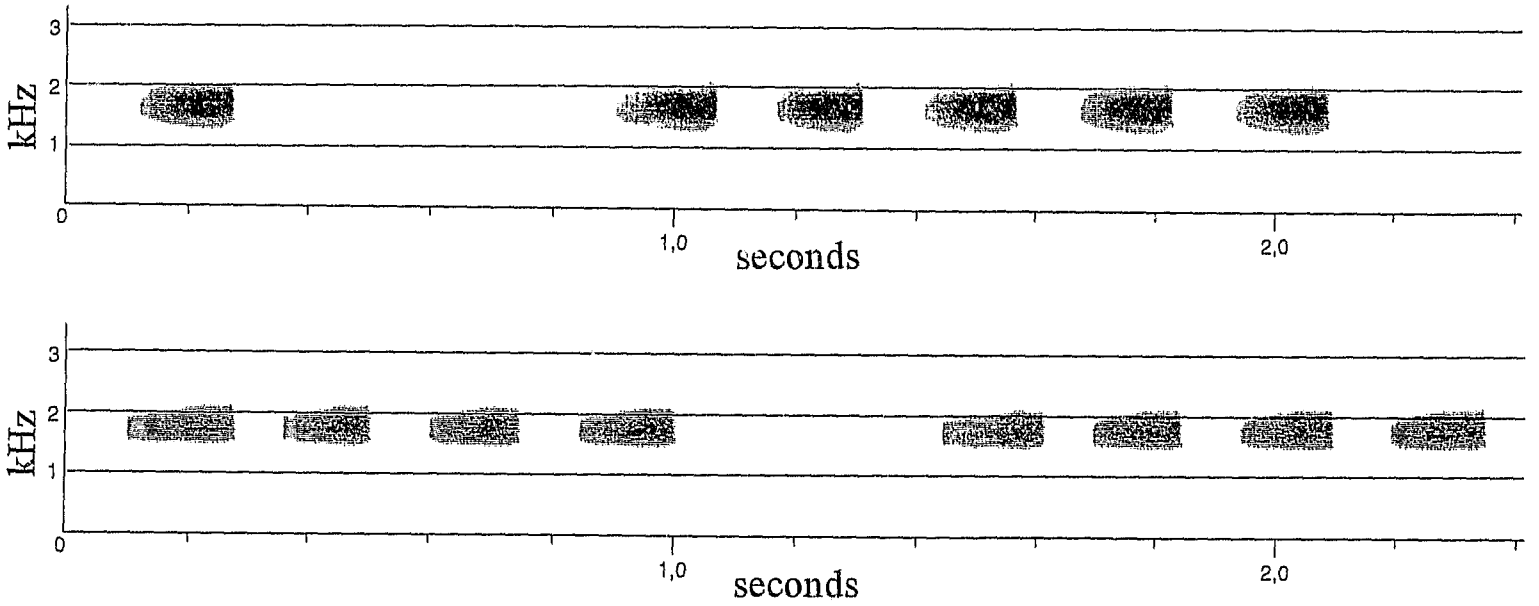

FIGURE 22. Grouping of calls within the call bout of *B. poweri* from Loc.40 (Nampula).

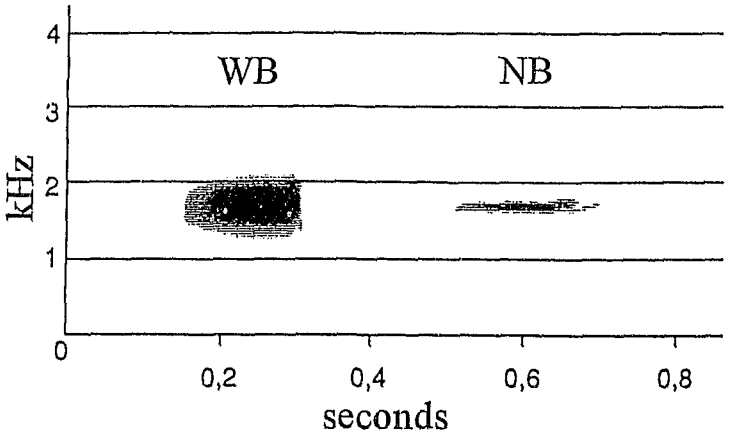

FIGURE 23. Wide (WB) and narrow band (NB) sonagrams of the advertisement call of *B. poweri* from Loc.40 (Nampula).

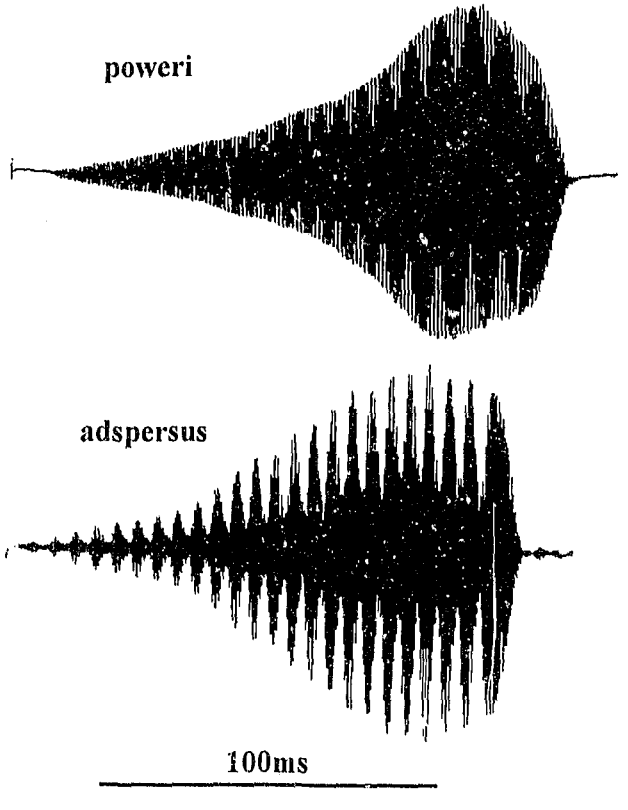

FIGURE 24. Oscillograms of the advertisement calls of *B. poweri* from Loc.40 (Nampula) and *B.a. adspersus* from Loc.42 (Pietersburg).

TABLE 43. Summary statistics of call bout and advertisement call variables, air temperature and body mass of a population of *B. poweri* sampled at Loc.40 (Nampula).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	39	6.89	1.21	0.38	4.04	10.28	6.82	1.44	1.01	17.59
Air temperature (°C)	40	25.5	1.5	0.5	22.3	23.8	25.7	1.1	-1.59	5.92
Bout variables:										
Bout duration (s)	47	8.2	6.1	1.8	3.0	29.7	8.2	8.7	3.34	58.85
Number of calls	47	30	16.3	4.7	10	74	25	20	2.85	54.89
Call rate (min^{-1})	47	175	27	8	124	251	169	31	2.17	15.17
% grouped calls	47	99.6	1.26	0.36	94	100	100	0	-9.06	1.27
Call interval (s):										
group-group	35	0.743	0.166	0.055	0.500	1.100	0.762	0.256	0.68	22.37
in group	40	0.235	0.020	0.006	0.176	0.263	0.239	0.018	-2.79	8.38
Call variables:										
Dom. frequency (Hz)	40	1728	83	26	1557	1903	1727	85	0.06	4.77
Call duration (s)	40	0.140	0.012	0.004	0.111	0.160	0.141	0.016	-1.18	8.89

7. Group 6 (*Breviceps sp.*)

7.1 Introduction.

This undescribed species (Fig.25a&b) was collected at three localities, viz:, Loc.22 (Komatipoort 2), Loc.51 & 53 (St Lucia 1 & 3), and heard calling at Loc.18 (Jozini 2), Mlawula Nature Reserve, Swaziland, and at Hluhluwe Nature Reserve in KwaZulu/Natal (pers. comm. Mr M. Burgers).

It occupies a variety of habitats including closed canopy coastal forest, tall moist evergreen forest on the western slopes of the Lebombo mountains and dry closed woodland (Lowveld) in Swaziland and Mpumalanga. At St Lucia 1 & 3 it was heard calling with *B. mossambicus* but was restricted to dense undergrowth under trees whereas *mossambicus* called from more open areas.

Dorsal colouring varies from a olive brown to grey, or light pink in some Komatipoort specimens. Faint light markings in the form of an interocular bar, paravertebral patches and a row of three confluent dorsolateral blotches are often present, as are dark speckles: these are obscured in more darkly pigmented specimens. A black stripe from eye to axilla is present and the gular patch is mottled or uniformly black. A thin, light vertebral stripe is sometimes present, extending from the thorax to the vent where it intersects a light stripe passing from heel to heel. The ventral surface is immaculate white or speckled.

The presence of light markings makes it difficult to distinguish this species from the southern populations of *B. mossambicus* and *B.a. adspersus*. It also resembles these species in the relative lengths of fingers and toes and the size, shape and distribution of palmar and metatarsal tubercles.

7.2 Advertisement call structure.

The advertisement call is not pulsed and consists of a long, weak, high-pitched whistle, repeated at regular intervals during a bout of calling (Fig. 26, Table 44). The calls are not grouped within the call bout.

The mean dominant frequency of 3236Hz distinguishes this species from all other *Breviceps spp.* sampled in this study. Because of the absence of amplitude modulation, sidebands are not developed in the narrow band spectrogram (Fig.26, NB).

Dominant frequency has the lowest coefficient of variation, while call duration is more variable than in other *Breviceps spp.*

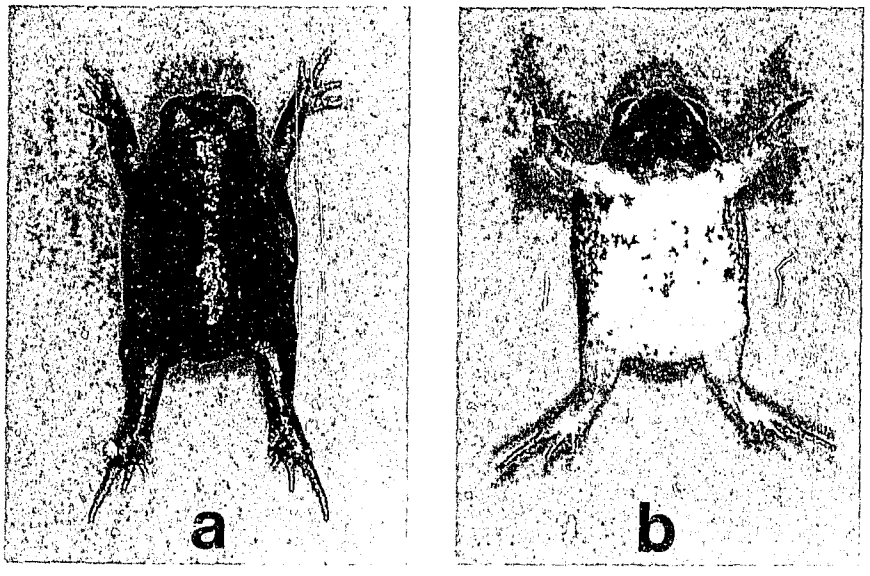

FIGURE 25. *Breviceps sp.* from Loc.51 (St Lucia 1): (a) dorsal; (b) ditto, ventral.

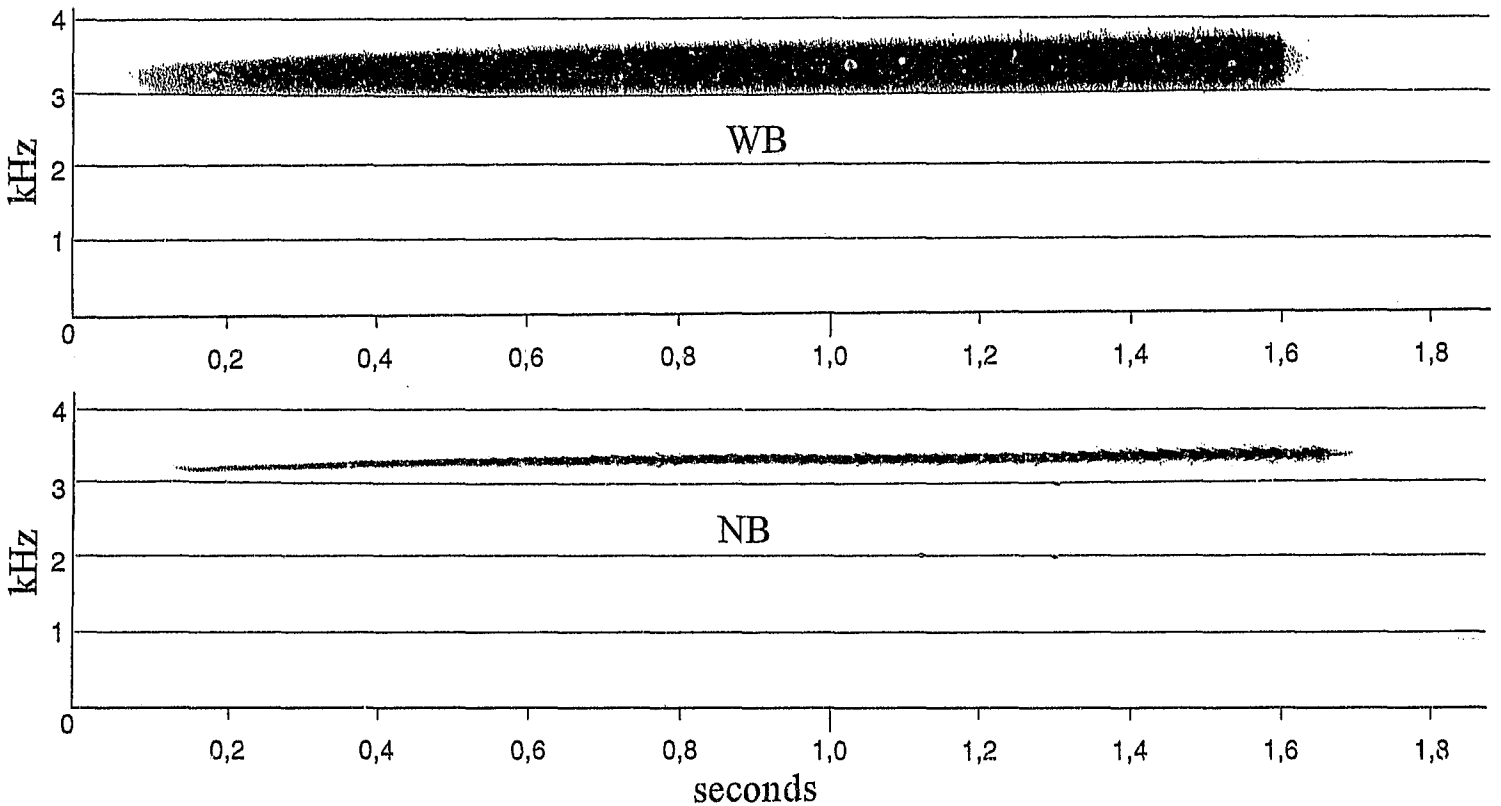

FIGURE 26. Wide and narrow band sonagrams of the advertisement call of a *Breviceps sp.* from Loc.51 (St Lucia 1).

TABLE 44. Summary statistics of call bout and advertisement call variables, air temperature and body mass of an undescribed *Breviceps sp.* from Loc.18 (Jozini 2), Loc.22 (Komatipoort 2), and Loc.51 & 53 (St Lucia 1 & 3).

Variables	N	Mean	SD	95%CI of mean	Min	Max	Median	Inter-quartile range	Std skew-ness	Coeff of var
Body mass (g)	8	2.02	0.16	0.11	1.76	2.27	2.01	0.21	-0.04	7.89
Air temperature (°C)	18	19.3	2.2	1.0	13.7	22.2	19.8	1.7	-2.34	11.33
Bout variables:										
Call rate (min^{-1})	13	23.3	8.6	4.7	6	36.4	23.1	6.4	-0.70	36.60
Call variables:										
Dom. frequency (Hz)	20	3237	144	63	2755	3468	3236	165	-3.36	4.46
Call duration (s)	20	1.183	0.344	0.151	0.600	1.983	1.065	0.479	1.24	29.12

B. MORPHOLOGY

1. Introduction.

Most of the specimens described below are adult males which were calling when collected. In Section A, these individuals were assigned to various species taxa on the basis of advertisement call structure. It is now necessary to describe the morphological variation present in the samples to determine the accuracy of existing species descriptions and diagnoses. A brief account of the taxonomy and distribution of the species dealt with in this study is given in the Introduction to this thesis (2.1 - 2.5) and the taxonomic implications of the morphological analysis are dealt with in the Discussion (2 and 3).

The diagnostic morphological characters identified by Poynton (1964), Poynton & Broadley (1985) and Lambiris (1989), as well as certain other potentially useful characters, are examined. A few explanatory notes concerning these morphological characters are necessary before proceeding with the description of the specimens.

Finger notation:

Poynton & Broadley (1985) use the finger notation 2 (inner) to 5 (outer), which is based on the assumption that the first digit has been lost. However, an alternative view holds that reduction in the number of digits occurs postaxially and that the fifth or outer digit has been lost (Duellman & Trueb, 1985). In order to avoid confusion the notation used by Poynton & Broadley is followed in this study.

Metatarsal tubercles:

The relative size and shape of the inner (larger) and outer (smaller) metatarsal tubercles, the degree of fusion between them and the angle between the base of the inner tubercle and the axis of the longest toe, were used as diagnostic characters by earlier workers such as Power (1926) and Parker (1934). Later authors have discarded most of these, but the degree to which the tubercles are fused is still used, eg. to distinguish *poweri* from *adspersus* and *mossambicus* (Poynton & Broadley, 1985).

In this study, a *cleft* refers to a deep division which extends to the base of the tubercles, completely separating them, while a *groove* is shallower and does not completely separate the tubercles. The groove is sometimes quite shallow, forming a mere undulation between the apices of the tubercles. Tubercles which are so closely fused that they cannot be distinguished from one another are said to be *united.*

Tubercles below the fingers & toes:

Two types of tubercle are distinguished:

subarticular tubercles - located below the joints of the digits.

subdigital tubercles - located between the subarticular tubercles

The latter may occupy a central position between two subarticular tubercles, or may lie closer to one or other of the subarticular tubercles, and sometimes are not distinguishable at all. Subdigital tubercles are therefore unreliable anatomical landmarks.

Relative length of fingers & toes:

The length of a digit relative to the subarticular tubercles of an adjacent digit, is often used as a diagnostic character. In this respect, the term:

reaches means the tip of a digit lies opposite the subarticular tubercle of the adjacent digit, between its proximal and distal margins.

barely reaches means the tip reaches the level of the proximal margin of the subarticular tubercle of the adjacent digit.

falls just short means the tip passes a point midway between the proximal and distal subarticular tubercles of the adjacent digit, but does not quite reach the distal tubercle.

falls far short means the tip extends beyond the level of the proximal subarticular tubercle of the adjacent digit, but not beyond the midway point.

With reference to the outer toe:

falls just short means that the apex of the outer toe is separated from the basal tubercle of the fourth toe by a distance which is less than the width of the basal tubercle of the fourth toe.

falls far short means that the apex of the outer toe is separated from the basal tubercle of the fourth toe by a distance greater than the width of the basal tubercle of the fourth

toe.

Colour and markings:

Light paravertebral and dorsolateral patches are present in several *Breviceps* species. While their position is relatively constant their colour and prominence varies greatly, even at the population level: in some individuals the patches are very distinct while in others they are so faint that they can hardly be seen. This is due to the degree of contrast between the colour or shade of the patch and that of the surrounding area. Sometimes the patches are rendered more conspicuous by the presence of black spots and speckles along their edges which form more or less continuous dark borders (Fig.2a), while in highly melanistic individuals, an excess of dark pigmentation tends to obscure lighter markings (Fig.27a-d).

Reduction in the prominence of light patches is sometimes more advanced in the paravertebral patches than the dorsolateral patches, ie. individuals in which the paravertebral patches have almost disappeared, still possess relatively distinct dorsolateral patches. At the species level, *B. poweri* has well developed dorsolateral patches while paravertebral patches, though present, are very indistinct (Fig.6a; Minter 1997:14-15). This suggests that dorsolateral patches are more important than paravertebral patches with regard to crypsis.

Another factor which influences the diagnostic value of these light patches, is the effect of preservatives on colour and markings. A comparison of preserved specimens (stored in darkness for 5-10 years) with colour photographs taken before preservation demonstrates the following effects of 70% ethanol (Fig.28a-d):

* dark brown fades to light brown, causing dark spots/speckles to become more prominent.
* orange becomes pink or, more often, cream or off-white.
* markings such as the interocular bar which, in life, are distinct due to bright colouration, become indistinct.

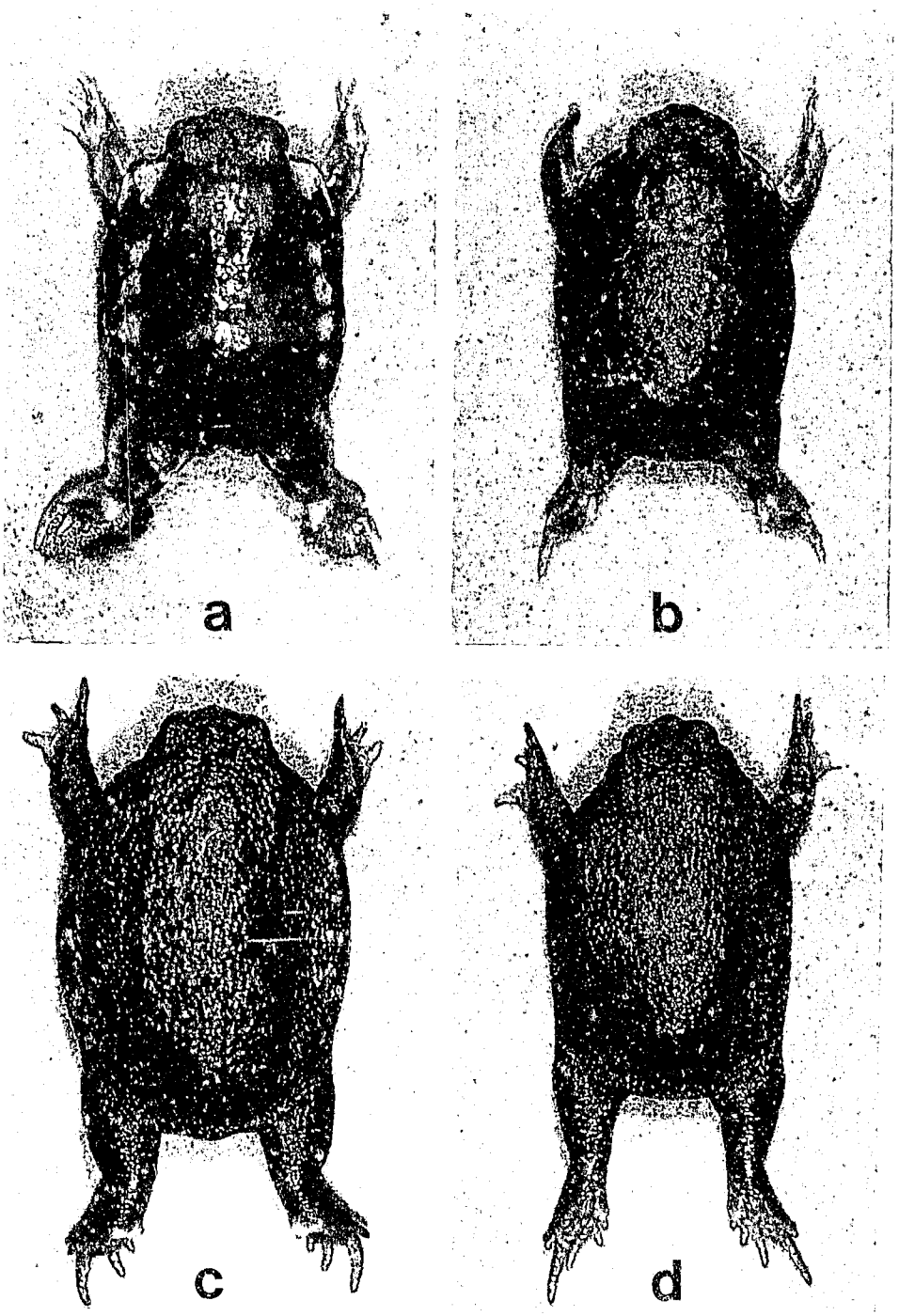

FIGURE 27. (a) *B.a. adspersus* male from Loc.38 (Mutare 1); (b) darkly pigmented *B.a. adspersus* male from Loc.38 (Mutare 1); (c) *B. verrucosus* male from Loc.12 (Harding), showing *tympanifer* markings partly obscured by pigmentation; (d) darkly pigmented *B. verrucosus* male from Loc.12 (Harding).

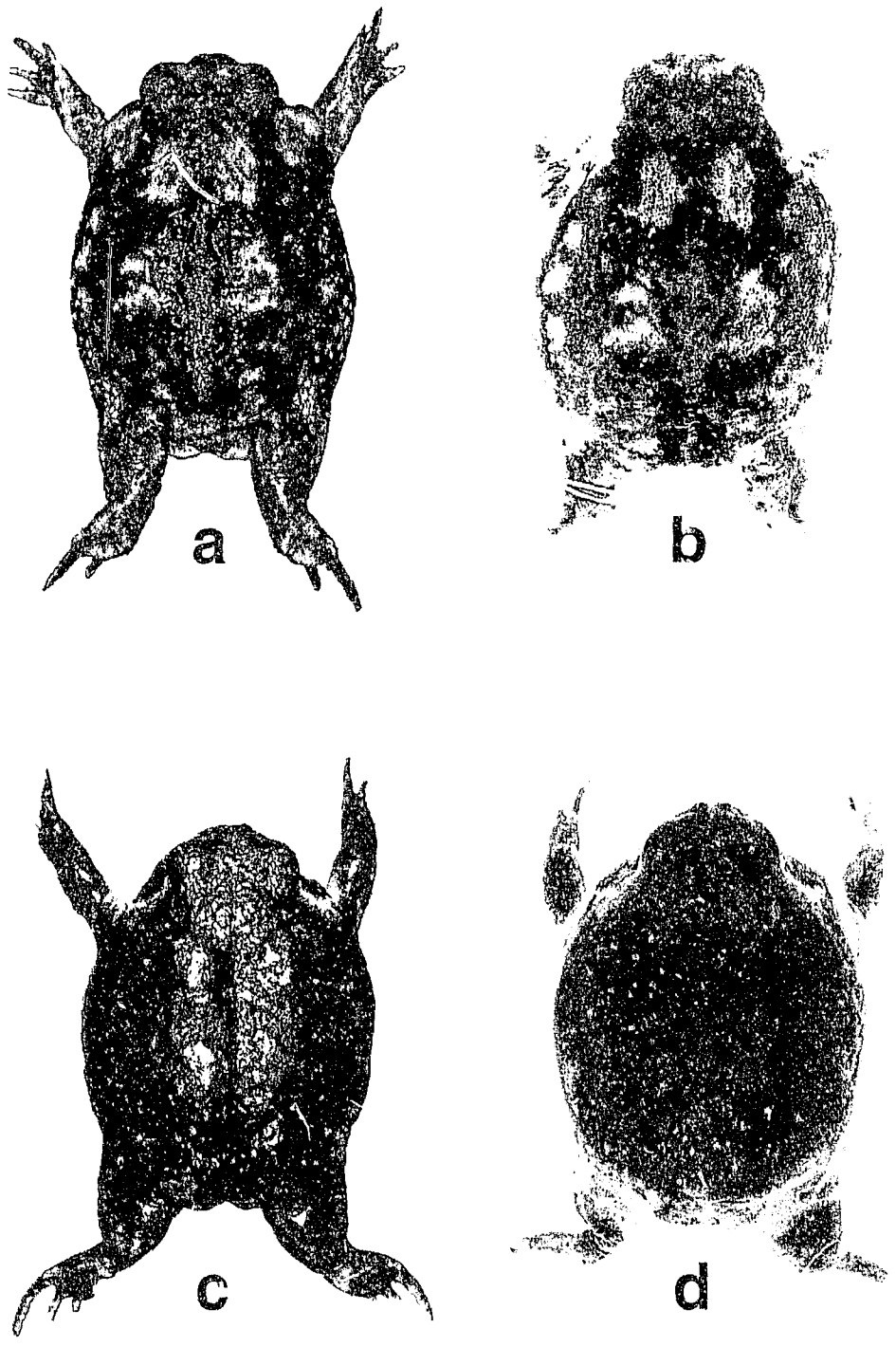

FIGURE 28. (a) *B.a. adspersus* male from Loc.42 (Pietersburg), before preservation; (b) ditto, 8 years after preservation; (c) *B.a. adspersus* male from Loc.38 (Mutare 1), before preservation; (d) ditto, 8 years after preservation.

The presence of paravertebral patches in an individual may therefore go undetected if:

* the patches are so indistinct that they are overlooked.
* they are obscured by dark pigmentation.
* they have been rendered less conspicuous by the preservative.

Thus the *adspersus X mossambicus* hybrids characterised by Poynton & Broadley (1985) as having dorsolateral patches and lacking paravertebral patches may represent *adspersus* individuals in which the paravertebral patches are very indistinct, obscured by darker pigmentation or bleached by preservative. Since the genetic basis of these markings is unknown, and experimental cross-matings have not been performed, invoking hybridisation to explain the variation, is purely speculative.

In this morphological study, unless otherwise stated, the colour of preserved specimens is described.

2. *Breviceps adspersus* Peters 1882

2.1 The status of *B.a. adspersus*: hybridisation with *mossambicus*

Poynton & Broadley (1985) maintain that *adspersus* and *mossambicus* are distinct species which hybridise extensively in sympatry. Relatively "pure" *adspersus* occurs in the southwest (Okavango area), while "pure" *mossambicus* is restricted to the northeast (northern Mozambique). In the "hybrid zone", individuals possess various combinations of *adspersus* and *mossambicus* characters.

Although these authors identify several diagnostic characters for each species, they use only one, the light dorsal and lateral markings, as an index of hybridisation in the hybrid zone. With regard to this character they define three patterns:

paravertebral and dorsolateral patches present	: *adspersus* pattern
paravertebral and dorsolateral patches absent	: *mossambicus* pattern
only dorsolateral patches present	: hybrid pattern

The presence of paravertebral patches in an individual may therefore go undetected if:

* the patches are so indistinct that they are overlooked.
* they are obscured by dark pigmentation.
* they have been rendered less conspicuous by the preservative.

Thus the *adspersus X mossambicus* hybrids characterised by Poynton & Broadley (1985) as having dorsolateral patches and lacking paravertebral patches may represent *adspersus* individuals in which the paravertebral patches are very indistinct, obscured by darker pigmentation or bleached by preservative. Since the genetic basis of these markings is unknown, and experimental cross-matings have not been performed, invoking hybridisation to explain the variation, is purely speculative.

In this morphological study, unless otherwise stated, the colour of preserved specimens is described.

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Although these authors identify several diagnostic characters for each species, they use only one, the light dorsal and lateral markings, as an index of hybridisation in the hybrid zone. With regard to this character they define three patterns:

paravertebral and dorsolateral patches present	: *adspersus* pattern
paravertebral and dorsolateral patches absent	: *mossambicus* pattern
only dorsolateral patches present	: hybrid pattern

All three patterns are generally present in series of specimens taken from different localities across the hybrid zone, ie. the hybrid pattern does not appear to have become stabilised within any one population. Also, the samples do not show a "simple east to west shift in the preponderance of one or other form, although there is a relatively higher representation of the *adspersus* pattern to the west".

Skin texture, i.e. granular or smooth, a character included in Peters' (1882) description of *adspersus* and used as a diagnostic character by Poynton (1964) and Lambiris (1989) is omitted, "because variation in both forms makes this feature valueless". Similarly, the light vertebral and heel to heel lines are said to have "little diagnostic significance".

The length of the outer finger is also apparently too variable to be of diagnostic value in identifying hybrids. Even the "pure" populations of the two species overlap in respect of this character: in the nine *adspersus* specimens from Caprivi (Okavango area) the outer finger only just passes the proximal subarticular tubercle of the fourth finger, but in two specimens it almost reaches the distal tubercle, while in the northern Mozambique specimens of *mossambicus* the third finger reaches the distal subarticular tubercle of the fourth finger but sometimes falls short in mainland material.

Another distinguishing character noted by Poynton & Broadley in their diagnoses for *adspersus* and *mossambicus*, is the pattern of pigmentation of the gular region: a single, dark gular patch characterises "pure" *mossambicus* while in "pure" *adspersus* the dark patch is divided in two by an immaculate, white midventral band. Although this character appears to be quite consistent in the "pure" populations of the two species, Poynton & Broadley do not use it to identify hybrids because "it is variable and sometimes indefinite". They note that "specimens from the hybrid zone with *adspersus* markings tend to have a single patch". For example, a " series of 15 specimens from Bulawayo all show the *adspersus* pattern" but possess an undivided gular patch: a "pure" *mossambicus* character outside of the hybrid zone. However, since these specimens do not show the "typical" *mossambicus* or hybrid patterns of dorsal markings, the authors assign them to *adspersus*.

It is not clear why the identification of the hybrids should be restricted to this single character. Surely the presence, in one individual, of any combination of the diagnostic characters of two species, indicates that the individual is a hybrid, and it should be identified unequivocally as such.

2.2 Material examined (103 specimens)

B.a. adspersus (68 specimens)

Loc 14 (Pietersburg)	44 males
Loc. 14 (Houtbosdorp 1)	1 male
Loc. 28 (Lydenburg 2)	1 male
Loc. 13 (Hoedspruit)	7 males
Loc. 57 (White River)	1 male
Loc. 21 (Komatipoort 1)	4 males
Loc. 29 (Manzini)	2 males
Loc. 3 (Burchenough Bridge)	2 males
Loc. 38 (Mutare 1)	6 males

B.a. pentheri (13 specimens)

Loc. 7 (Grahamstown)	11 males
Loc. 41 (Paterson)	1 male
Loc. 1 (Addo)	1 male

Populations with unusual variation in advertisement call structure (22 specimens) (see Results, 2.4.1)

Loc. 16 (Ingwavuma)	14 males
Loc. 49 (Sihangwane 4)	3 males
Loc. 50 (Sihangwane 5)	5 males

2.3 Morphology of specimens assigned to *B.a. adspersus*

2.3.1 Diagnostic characters.

The characters listed below are regarded by Poynton & Broadley (1985) as diagnostic of "pure" *adspersus*.

* *Outer finger usually falling far short of distal tubercle of fourth finger.*
Only seven of the 68 specimens fit this description, viz: four from Pietersburg, one from Hoedspruit and the single specimens from Houtbosdorp and White River. The remainder have longer outer fingers which reach the distal subarticular tubercle in one specimen, barely reach it in 12, and fall just short in 40 individuals.

Thus, in respect of this character, the majority of specimens fall within the diagnostic range attributed by Poynton & Broadley to *mossambicus* rather than *adspersus*.

* *Inner and outer metatarsal tubercles separated by a cleft.*
B. adspersus and *B. mossambicus* share this character which serves to separate them from *B. poweri* in which the tubercles are usually united. In the Pietersburg sample, 29 have a cleft between the tubercles while 15 have a groove. In the remainder of the *adspersus* specimens a cleft is present in 12 and a groove in 12. Four of the specimens described above (from Pietersburg, Manzini and Mutare) have tubercles which are united on one foot and separated by a groove on the other.

Thus only 41 of the 68 specimens examined conform to this character state.

* *A series of light paravertebral and dorsolateral patches present.* (Fig.2a)
Light paravertebral patches are clearly visible in 56 specimens, indistinct in the Lydenburg specimen, two Komatipoort, one Manzini and seven Zimbabwe specimens and absent in one Zimbabwe specimen. Light dorsolateral patches are distinct in 64 specimens and indistinct in the Lydenburg and three Zimbabwe specimens. Thus only one specimen fits Poynton & Broadley's definition of a "typical hybrid": all oth-

ers conform to their "*adspersus* pattern".

* *Gular region with a pair of marbled to freckled patches, separated by an immaculate midventral band.*

Thirteen Pietersburg individuals have a uniformly darkened gular patch which is partly to entirely divided by a median white band in six specimens; 31 have a marbled patch, divided medially in 15.

The specimens from Zimbabwe and Lydenburg have a uniformly darkened, undivided gular patch, three from Hoedspruit are uniformly darkened, with one divided medially, and the remainder of this and other populations sampled (13 specimens) have a marbled and undivided gular patch.

In total, 22 of 68 specimens exhibit this diagnostic character.

* *Dark infraorbital patch tending or actually extending to base of arm.*

The distinction between *adspersus* and *mossambicus* with regard to this character is not clear unless one refers to the corresponding character state in the diagnosis for *mossambicus*, which states: "Dark infraorbital patch extending to base of arm, in mainland material usually broadly continuous with gular patch". The alternative character state, ie. applicable to *adspersus*, is that the dark infraorbital and gular patches are separated by a light line which runs from the eye downwards in front of the infraorbital patch, to the base of the arm. In the nine Caprivi specimens of "pure" *adspersus* examined by Poynton & Broadley, the infraorbital patch is completely separated from the dark gular patch in seven specimens, and tenuously separated in two.

Most specimens examined in this study have the infraorbital patch separated from the gular patch, except for five Pietersburg individuals and the single, darkly pigmented Houtbosdorp specimen, ie. 62 of 68 specimens exhibit this diagnostic character.

2.3.2 Other morphological characters: (Fig.2a&b)

Tympanum - faintly distinguishable in five Pietersburg & one Komatipoort specimen; not distinguishable in the rest.

Vertebral line - present but indistinct in eight Pietersburg, one Houtbosdorp, four Hoedspruit and three Komatipoort specimens.

Heel to heel line - distinct in seven Pietersburg and one Houtbosdorp specimen; indistinct in 23 Pietersburg, five Hoedspruit, one White River, three Komatipoort, one Manzini, and two Zimbabwe specimens.

Urostylar patch - distinct in three Pietersburg, one Hoedspruit, one White River and one Komatipoort specimen; indistinct in 22 Pietersburg, one Hoedspruit, one Komatipoort and one Manzini specimen.

Abdomen - immaculate in most specimens; lightly freckled in eight Pietersburg, one Lydenburg and one Zimbabwe specimen.

Proximal subarticular tubercle of 4th finger - divided in the specimen from White River; single in the rest.

Skin texture - The dorsal surface is granular to densely granular in most specimens, with the granulations more concentrated laterally in some. Unlike *verrucosus* and *sylvestris*, the granules are not deeply pitted. About 5-6 openings of glands are visible on the surface of each granule but are not more concentrated here than on the surrounding skin. The larger granules are usually darkly pigmented. The dorsum is completely smooth in seven Pietersburg, two Hoedspruit, one White River, one Komatipoort and seven (most) Zimbabwe specimens. The abdomen is smooth in all specimens.

2.4 Status and distribution of *B.a. pentheri*

B. pentheri was described from the eastern Cape (apparently Grahamstown) in 1899 and Parker (1934) later synonymised *B. parvus* from the eastern escarpment of Natal with this species. Poynton (1964) changed the status of *pentheri* from a full species to that of a subspecies of *B. adspersus* and gave its distribution as "Upper eastern plateau slopes of Natal, reaching sea level in the south-eastern Cape". He notes that "This form appears to lie on the fringe of *adspersus* on the upper eastern plateau slopes, the

line of division apparently coinciding with the 13°C mean July isotherm in Natal".

The results of the advertisement call analyses in the present study suggests that the populations occupying the upper eastern plateau slopes are neither *adspersus* nor *pentheri*, but should instead be referred to *mossambicus*.

Thus the diagnostic characters for *pentheri* noted by Poynton (1964) are based on specimens representing two different species. The morphological descriptions below are therefore restricted to specimens from the Eastern Cape which are assigned to *pentheri* on the basis of advertisement call structure.

2.5 **Morphology of specimens assigned to *pentheri*** (13 specimens)

2.5.1 Diagnostic characters (Poynton, 1964) (Fig.2c&d)

* *Tympanum invisible.*
The tympanum is not distinguishable in any of the specimens.

* *Outer toe not reaching basal tubercle of fourth, falling far short of second.*
It is not clear whether Poynton means "second" tubercle of the fourth toe or "second" toe. If he means the former, the observation is pointless because, as he has already stated, the outer toe does not even reach the level of the basal tubercle; the latter possibility is equally unlikely since the outer toe reaches, at least, the level of the basal tubercle of the second toe in *adspersus, poweri, mossambicus* and the *sp. nov.* and passes this level, even extending beyond the tip of the second toe, in some *verrucosus* and *sylvestris* specimens.

In the 13 *pentheri* specimens examined, the outer toe falls far short of the basal tubercle of the fourth in two specimens and just short in 11.

* *Dorsal surface usually very rough and porous.*
The dorsal surface is densely granular in four specimens and granular in nine: some

specimens are smoother medially, and others laterally, but none is entirely smooth. The granules are large and prominent but fairly widely spaced and resemble those of *B.a. adspersus* as described in 2.3.2 above. The specimen from Addo is covered in unusually large, almost warty granules.

* *Ventral surface fairly smooth to granular.*

Four specimens are densely granular over the entire gular region, and in three, the g[illegible] are restricted to the sides of the gular patch. The abdominal surface is smooth in all specimens.

* *Dorsal markings varying from a light median band with deeply serrated edges to a row of paravertebral light patches, to a uniform dark brown or grey.*

An interocular bar is present in 10 specimens and indistinct in three. Distinct paravertebral patches are present in five specimens, indistinct in seven and absent in one; in two specimens the scapular pair extend forward as far as the interocular bar. The dorsolateral patches are distinct in 11 and indistinct in two specimens. A serrated median band was not observed, but is present in specimens from the eastern escarpment, here referred to *mossambicus*. The dorsal markings are too variable to qualify as a diagnostic character, ie. "the essential characters by which the species is distinguished from its congeners" (Mayr, 1969).

* *Throat marbled to heavily darkened.*

The gular patch is uniformly dark in seven specimens, darkened with lateral marbling in t[illegible]nd entirely marbled in three. The patch is not divided by a median white stripe. All specimens thus conform to the diagnosis in respect of this character.

* *Chest and belly with dark flecks.*

The pectoral region is heavily freckled in four specimens, moderately in four and lightly freckled in five. The abdomen is heavily freckled in three specimens, moderately in seven and lightly freckled in three. All specimens thus conform to the diagnosis in respect of this character.

2.5.2 Other morphological characters

Outer finger - reaches the distal subarticular tubercle of the 4th finger in 10 specimens, barely reaches it in one and falls just short in two. In this respect the sample conforms to the character state diagnostic of *mossambicus* rather than *adspersus*.
Vertebral and heel to heel lines - absent.
Urostylar patch - absent.
Infraorbital patch - separated from the dark gular patch by a light band in 12 specimens, continuous with it in one.
Proximal subarticular tubercle of 4th finger - not divided.
Inner and outer metatarsal tubercles - separated by a cleft in eight specimens and a groove in five.

2.6 Morphology of populations with unusual variation in advertisement call structure: Loc.16 (Ingwavuma) and Loc 49 & 50 (Sihangwane 4 & 5) (22 specimens)

2.6.1 Diagnostic characters.

These populations exhibit a high degree of variation in their advertisement call structure which suggests that they may contain hybrids. The three samples are combined because of their close proximity and similarities with regard to call structure, habitat and body size. The diagnostic characters for "pure *adspersus*" are examined below.

* *Outer finger usually falling far short of distal tubercle of fourth finger.*
Only five specimens fit this description. The remainder have longer outer fingers which barely reach the distal subarticular tubercle in five specimens, and fall just short of it in 12. Thus, in respect of this character, the majority of specimens fall within the range diagnostic of *mossambicus*, as was the case in the other *adspersus* specimens examined above.

* *Inner and outer metatarsal tubercles separated by a cleft.*

Most (29) specimens have a cleft between the tubercles while one has a groove. Almost all specimens thus conform to the diagnosis in respect of this character.

* *A series of light paravertebral and dorsolateral patches present.*

Light paravertebral patches are distinct in 14 specimens, and indistinct to very indistinct in 8. Light dorsolateral patches are present in 20 specimens, indistinct in one and absent in one specimen. Almost all specimens thus conform to the diagnosis in respect of this character.

* *Gular region with a pair of marbled to freckled patches, separated by an immaculate midventral band.*

Most (19) individuals have a marbled/freckled gular patch while three have a uniformly darkened patch which is divided posteriorly by a median white stripe, in one. Thus most specimens do not conform to the diagnosis in respect of the median white band.

* *Dark infraorbital patch tending or actually extending to base of arm.*

A white stripe separates the infraorbital patch from the gular patch in 20 specimens, while the patches are tenuously connected in two. Almost all specimens thus conform to the diagnosis in respect of this character.

2.6.2 Other morphological characters:

Tympanum - very faintly distinguishable in eight specimens; not distinguishable in 14.

Vertebral line - distinct in 10 specimens, indistinct in seven and absent in five. The line extends to the head in 12 specimens and is present posteriorly in five.

Heel to heel line - distinct in five specimens, indistinct in six and absent in 11.

Urostylar patch - absent in 20 specimens and poorly developed in two.

Abdomen - immaculate white in 20 specimens; lightly freckled in two.

Proximal subarticular tubercle of 4th finger - single in 21 specimens, divided in one.

Skin texture - dorsal surface granular in 12 specimens and smooth in 10: granulations are more concentrated laterally in two of these specimens. Ventral surface smooth.

3. *Breviceps mossambicus* Peters 1854

3.1 Taxonomic status

Peters' description of *B. mossambicus* was based on specimens from Mozambique Island and Sena on the mainland. Poynton & Broadley (1985) base their description of "pure *mossambicus*" on material from the Island population. Their suggestion that *mossambicus* hybridises extensively with *adspersus* is discussed in 2.1 above.

The morphological descriptions of the populations referred to *mossambicus* follow the same sequence and grouping as in the analysis of their advertisement calls (Section A). Because of the large number of populations sampled, a synopsis of the morphological data for *mossambicus* is presented in 3.4 below.

3.2 Material examined (166 specimens)

Northern Mozambique (46 specimens)	
Loc. 36 (Mozambique Island)	17 males, 3 females
Loc. 26 (Lumbo)	5 males
Loc. 40 (Nampula)	21 males
Southern Mozambique and KwaZulu/Natal coastal plain (76 specimens)	
Loc.30 (Maputo)	4 males
Loc.23 (Lake Kosi)	3 males
Loc.46 & 47 (Sihangwane 1&2)	21 males, 1 female
Loc.51 & 52 (St Lucia 1&2)	5 males
Loc.37 (Mtunzini)	14 males, 1 female
Loc.6 (Eshowe)	5 males
Loc.17 & 19 (Jozini 1&3)	10 males
Loc.35 (Mkuze)	12 males

Escarpment of KwaZulu/Natal, Swaziland, Mpumalanga and Northern Province (44 specimens)

Loc.2 (Belfast) & Loc.27 (Lydenburg 1)	15 males
Loc.31, 32 & 33 (Mbabane1, 2 & 3)	5 males
Loc.56 Wakkerstroom) & Loc.43 (Piet Retief)	3 males
Loc.8 (Graskop 1)	18 males
Loc.11 (Haenertsburg 2)	3 males

3.3 Morphology of specimens assigned to *mossambicus* (Figs.3a-d, 17a-f)

3.3.1 Populations from northern Mozambique: Loc.36 (Mozambique Island), Loc.26 (Lumbo) & Loc.40 (Nampula). (46 specimens)

3.3.1.1 Diagnostic characters (Poynton and Broadley 1985)

** Outer finger reaching or nearly reaching distal subarticular tubercle of fourth finger.*

In all specimens the outer finger falls just short of the distal subarticular tubercle of the fourth finger. All specimens thus conform to this diagnostic character state.

** Inner and outer metatarsal tubercles separated by a cleft.*

The tubercles are separated by a deep cleft in 16 Island and 14 mainland specimens and a shallow groove in four Island and nine mainland specimens; and are united in three mainland specimens. The sample is more variable in respect of this character than is reflected in the diagnosis.

** No light paravertebral or dorsolateral patches.*

Light paravertebral and dorsolateral patches absent except for one Island specimen and two mainland specimens. In the latter, the dorsolateral patches are indistinct and are present only on one side of the body. Almost all specimens thus conform to the diagnosis in respect of this character.

* *Gular region with a single uniformly darkened to (less usually) marbled to freckled patch.*

In the Island sample this region is uniformly dark anteriorly and freckled posteriorly in 13 specimens, while in seven specimens the entire gular region is freckled; in the mainland sample, the gular region is uniformly darkened in all specimens. The variability present in the samples reduces the diagnostic value of this character.

* *Dark infraorbital patch extending to base of arm, in mainland material usually broadly continuous with gular patch.*

In the Island sample the infraorbital stripe is, with one exception, separated from the dark gular patch by a light stripe which extends from the upper jaw to the arm; in 18 mainland specimens the infraorbital patch joins the gular patch, is separated from it in four, while four specimens show a combination of these two character states. The variability present in the samples reduces the diagnostic value of this character.

3.3.1.2. Other morphological characters

Tympanum - not distinguishable.

Proximal subarticular tubercle of 4th finger - divided in two mainland specimens but only on one hand; single in the rest.

Outer toe - in all specimens it is reduced to a stub which falls just short of the basal subarticular tubercle of the fourth toe.

Interocular bar - present but very indistinct in 13 Island specimens and absent in seven; in the mainland sample it is barely discernable in 14 and absent in 12.

Vertebral line - in the Island sample a narrow, light vertebral line extends forward to reach the head in two specimens, is shorter and sometimes indistinct in 16 and absent in two; in the mainland sample it is short and indistinct in seven specimens and absent in 19.

Heel to heel line - present in all Island specimens but indistinct in 11; while in the mainland sample it is absent in 15 specimens, indistinct in nine and distinct in two. This character is too variable to be of any diagnostic value.

Urostylar patch - absent.

Skin texture - the dorsal surface is smooth in 44 specimens and slightly granular laterally in two. The ventral surface is smooth.

Colour and markings.

Island sample: dorsally, all specimens are uniform light brown, with conspicuous black spots (Fig.3a) that vary considerably in number and size; some individuals are almost immaculate while others, especially females, are heavily spotted and even blotched. Three individuals possess a few small, inconspicuous red patches. Fine, light lateral speckling is present in all specimens, but is usually inconspicuous. Ventrally, the abdomen is immaculate white in all Island specimens.

Mainland sample: dorsally, 24 specimens are dark brown to black (Fig.3c) while two from Lumbo are light brown. Indistinct dark spots scattered over the entire dorsum are present in 14 specimens while in 12 the background colour is so dark that the spots, if present, are not discernible. A red or pink suffusion is present in 21 specimens, spread across the entire dorsum except in darker specimens where it is only visible laterally or is restricted to the light area behind the eyestripe. Five specimens show no red colouring. Fine, light lateral speckling is present in 13 specimens but usually indistinct, and absent in 13. Ventrally, the abdomen is immaculate white (Fig.3b&d) in 24 specimens and lightly freckled in two.The most obvious difference between Island and Mainland samples lies in the overall colour rather than in their markings

3.3.2 Southern Mozambique and KwaZulu/Natal coastal plain

Populations from this area differ markedly in appearance from those of *mossambicus* in northern Mozambique, as they possess a number of "*adspersus* characters" such as light paravertebral and dorsolateral patches (Fig.17a-d) and a divided gular patch. However, on the basis of advertisement call structure, they can be clearly distinguished from *adspersus* in areas of parapatry, and are indistinguishable from the northern Mozambique populations of *mossambicus*. These populations are therefore referred to *mossambicus* (see Section A, 3.8). Unfortunately, this eliminates the single consistent morphological difference between *mossambicus* and *adspersus*, viz: the presence or absence of light dorsal patches.

A number of morphological characters are examined to determine their variability and assess their potential diagnostic value

3.3.2.1 Loc.30 (Maputo), Loc.23 (Lake Kosi) and Loc.46&47 (Sihangwane 1&2) (29 specimens)

These populations occupy similar habitats and are indistinguishable in terms of overall appearance, advertisement call structure and behaviour. The samples are therefore combined in the following description.

* *Tympanum*. Indistinguishable in 28 specimens, faintly visible in one.
* *Length of outer finger*. The outer finger falls far short of the distal subarticular tubercle in 14 specimens, just short in 14 and barely reaches the distal subarticular tubercle in one.
* *Proximal subarticular tubercle of 4th finger*. This is undivided in 25 specimens, divided on one hand in three and on both hands in one specimen.
* *Inner and outer metatarsal tubercles*. These are separated by a deep cleft in 27 specimens and by a groove in two.
* *Length of outer toe*. This forms a stub which falls far short of the basal subarticular tubercle of the fourth toe in 19 specimens and just short in 10.
* *Light interocular bar*. This is distinct in 10 specimens, indistinct in 17 and absent in two. In 4 specimens the bar has a black posterior border.
* *Light paravertebral patches*. These are distinct in 25 specimens and indistinct in four. The pair of large, elongated scapular patches are partly or entirely edged in black in 22 specimens. Two to four pairs of smaller, light patches are located posterior to the scapular patches. In 16 specimens they are outlined in black, laterally, forming a slightly, to deeply notched lateral border to the group of patches. In some individuals the black edging extends medially to the midline clearly separating the patches from one another and creating a pattern of alternating broad (light) and narrow (dark) transverse bars. In others, the patches are not clearly separated and may fuse completely to form a single, large, posterior patch.

* *Light dorsolateral patches*. These are distinct in 25 specimens and indistinct in four. The row of three to four rounded patches usually has a dark lateral (ventral) border, which sometimes extends medially (dorsally), surrounding and separating the individual patches. In other specimens the patches lack dark edges and may be partially or completely united.

* *Vertebral line*. This extends to the head and is distinct in 26 specimens and indistinct in three.

* *Heel to heel line*. This is distinct in 11 specimens and indistinct in 18.

* *Urostylar patch*. Absent.

* *Gular region*. In 26 males a median white stripe divides the dark gular patch into two parallel, longitudinal, dark bands. The gular patch is uniformly darkened in one male, mottled in another and immaculate white in the female specimen.

* *Dark infraorbital patch*. In 24 specimens, it is separated from the dark gular patch by a white stripe which extends from the upper jaw to the arm, but in five specimens the white stripe does not reach the arm and the two dark patches are tenuously connected inferiorly.

* *Abdomen*. It is immaculate white in 24 specimens, while in five the abdomen is lightly stippled or freckled.

* *Skin texture*. The dorsum is entirely smooth in 25 specimens, while in four it is granular laterally and smooth medially. The ventrum is smooth in all specimens.

* *Colour and markings*. There is a high degree of colour variation within these populations (Fig.29). The interocular bar varies from light brown to grey or white, the paravertebral patches are light brown in 19 and white in six specimens, and the dorsolateral patches are light brown in 13 and white in 12 specimens. In 19 specimens the paravertebral patches are surrounded to a varying degree, by bright, brick red spots or more extensive blotches.The rest of the dorsum medial to the dorsolateral patches is uniform light brown in 15, brown in six, dark brown in two, grey-brown in three and grey in one specimen; one specimen is light brown (dark brown in life) with numerous dark spots. Lateral (ventral) to the dorsolateral patches the colour is light brown in 10 specimens, brown in three, dark brown in 13 and grey-brown in three.

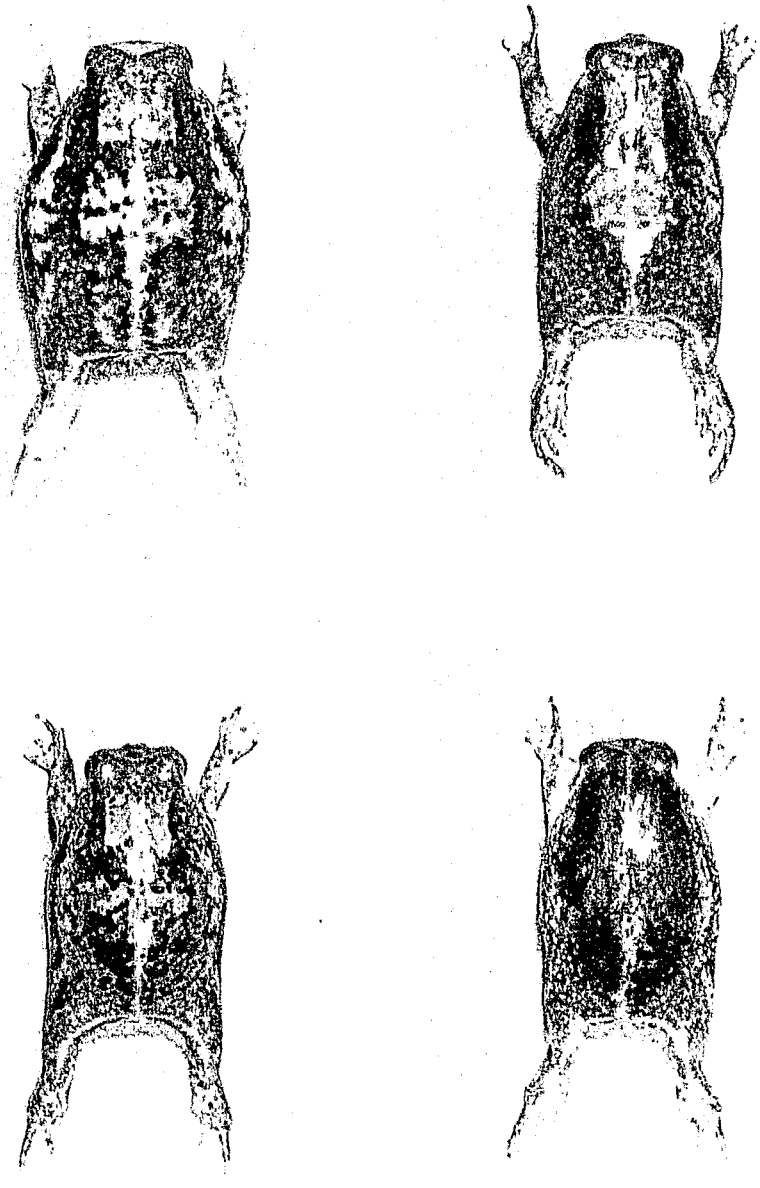

FIGURE 29. Variation in dorsal colouration of *B. mossambicus* males from Loc.47 (Sihangwane 2).

3.3.2.2 Loc.51 & 52 (St Lucia 1&2) (5 specimens)

* *Tympanum.* Not distinguishable.

* *Length of outer finger.* It falls just short of the distal subarticular tubercle of the fourth finger.

* *Proximal subarticular tubercle of 4th finger.* Not divided.

* *Inner and outer metatarsal tubercles.* Separated by a cleft.

* *Length of outer toe.* It falls just short of the basal subarticular tubercle of the fourth toe in four specimens and far short of it in one.

* *Light interocular bar.* It is distinct in four specimens and indistinct in one. The bar has a black outline in all specimens.

* *Light paravertebral patches.* They are distinct in four specimens and indistinct in one. The patches are outlined in black in all specimens.

* *Light dorsolateral patches.* Indistinct in two specimens and very indistinct in three.

* *Vertebral line.* Extends to the head:distinct in four specimens, very indistinct in one.

* *Heel to heel line.* This is distinct in four specimen and, very indistinct in one.

* *Urostylar patch.* Absent.

* *Gular region.* This is clearly divided into two dark longitudinal bands in one specimen, partly divided in one and indistinctly divided in two. One specimen has a uniformly darkened gular patch.

* *Dark infraorbital patch.* A white stripe separates this from the gular patch in four specimens while in one specimen the dark patches are tenuously connected.

**Abdomen.* Immaculate white.

* *Skin texture.* Both the dorsum and ventrum are smooth.

* *Colour and markings.* The interocular bar is light brown in four specimens and grey-brown in one; the paravertebral patches are light brown; the dorsolateral patches are slightly lighter brown than the surrounding area, and are therefore difficult to distinguish.. In four specimens the dorsum medial to the dorsolateral patches is light brown to tan with distinct black speckles concentrated around the paravertebral patches (Fig.17b), while in one specimen, almost the entire dorsum is light pinky red. Lateral (ventral) to the dorsolateral patches the colour is light brown in four specimens and darker brown in one.

3.3.2.3 Loc.37 (Mtunzini) (15 specimens; Fig.18c)

* *Tympanum.* This is not distinguishable in 13 specimens and faintly visible in two.
* *Length of outer finger.* It falls just short of the distal subarticular tubercle of the fourth finger in 10 specimens, far short in two, and barely reaches it in three.
* *Proximal subarticular tubercle of 4th finger.* Not divided.
* *Inner and outer metatarsal tubercles.* Separated by a cleft.
* *Length of outer toe.* It falls far short of the basal subarticular tubercle of the fourth toe in in seven specimens, just short in three, while five individuals show a combination of these two character states.
* *Light interocular bar.* It is distinct in nine specimens, indistinct in four and not visible in two.
* *Light paravertebral patches.* They are distinct in 12 specimens and indistinct in three. The patches have black borders in 10 specimens and in eight of these, a deeply notched lateral border.
* *Light dorsolateral patches.* They are present but lack a black outline and are therefore not as distinct as the paravertebral patches.
* *Vertebral line.* This is absent in nine specimens and present posteriorly in six. In two of these specimens the line is indistinct.
* *Heel to heel line.* Present in one specimen, indistinct in eight and absent in six.
* *Urostylar patch.* Absent.
* *Gular region.* This is uniformly dark in 13 specimens and marbled in two. None of these specimens has a divided gular patch.
* *Dark infraorbital patch.* A white stripe completely separates this from the gular patch in 12 specimens while in three specimens the dark patches are tenuously connected.
* *Abdomen.* Light freckling is present in eight specimens and moderate freckling in five, while two are immaculate white.
* *Skin texture.* The dorsum in all specimens is granular laterally and smooth medially. The ventrum is smooth.
* *Colour and markings.* The interocular bar is light brown to off-whi in 12 specimens and white in one; paravertebral patches are light brown (apricot in live speci-

mens - Fig.17c) to off-white in 13 specimens, white in one and greyish white in one; dorsolateral patches are light brown to off-white in nine specimens, pinkish in three, white in three and greyish white in one. In five specimens the dorsal surface medial to the dorsolateral patches is uniform dark brown, five specimens are light brown with dark speckles or spots, and five are dark grey-brown with dark speckles or spots. Lateral (ventral) to the dorsolateral patches the colour is dark brown in 10 specimens and very dark grey- brown in five, with distinct lateral marbling developed in two.

3.3.2.4 Loc.6 (Eshowe) (5 specimens; Fig.17d)

* *Tympanum.* Not distinguishable.
* *Length of outer finger.* The outer finger falls just short of the distal subarticular tubercle of the fourth finger.
* *Proximal subarticular tubercle of 4th finger.* These are not divided.
* *Inner and outer metatarsal tubercles.* These are separated by a cleft.
* *Length of outer toe.* It falls far short of the basal subarticular tubercle of the fourth toe.
* *Light interocular bar.* This is distinct in four specimens, and very indistinct in one. The bar has a black border in one specimen.
* *Light paravertebral patches.* These are distinct and bordered in black in four specimens and indistinct in one.
* *Light dorsolateral patches.* These are distinct in four specimens and indistinct in one. The patches are outlined in black in two specimens.
* *Vertebral line.* This is indistinct in three specimens, extending to the head in two. It is absent in the remaining two specimens.
* *Heel to heel line.* This is absent in four specimens and indistinct in one.
* *Urostylar patch.* This is present in three specimens and absent in two.
* *Gular region.* This region is uniformly darkened in four specimens and mottled in one.
* *Dark infraorbital patch.* In four specimens the infraorbital patch is tenuously connected to the gular patch, while in one, the white stripe completely separates them.
* *Abdomen.* This is lightly freckled in four specimens and immaculate white in one.

* *Skin texture.* The dorsum and ventrum are smooth.

* *Colour and markings.* The interocular bar is light brown in one specimen, cream in three and white in one; the paravertebral patches are light brown in one specimen, cream in three and white in one; the dorsolateral patches are light brown in one specimen, cream in three and white in one. The dorsal surface medial to the dorsolateral patches is light brown with dark speckles in one specimen, uniform dark brown in two and dark brown with dark spots in two. The area lateral (ventral) to the dorsolateral patches is dark brown.

3.3.2.5 Loc.17 & 19 (Jozini 1&3) (10 specimens)

* *Tympanum.* Not distinguishable.

* *Length of outer finger.* The outer finger falls just short of the distal tubercle of the fourth finger in eight specimens, far short of it in one, and barely reaches the distal tubercle in one.

* *Proximal subarticular tubercle of 4th finger.* This is not divided.

* *Inner and outer metatarsal tubercles.* These are separated by a deep cleft in seven specimens and by a shallow groove in three.

* *Length of outer toe.* The outer toe falls just short of the basal subarticular tubercle of the fourth toe.

* *Light interocular bar.* This is distinct in five specimens and indistinct in five. In two specimens the bar is outlined in black.

* *Light paravertebral patches.* These are distinct in six specimens, indistinct in three and not distinguishable in one. A broken black border surrounds the patches in five specimens.

* *Light dorsolateral patches.* These are distinct in nine specimens and indistinct in one. A black border is present in one specimen.

* *Vertebral line.* This is absent in seven specimens and present posteriorly, but indistinct, in three.

* *Heel to heel line.* Absent in eight specimens and present, but indistinct, in two.

* *Urostylar patch.* This is absent in eight pecimens and present, but indistinct, in two.

* *Gular region.* This is marbled in seven specimens and uniformly dark in three. In

four of these specimens the gular patch is partially or completely divided into two longitudinal bands.

* *Dark infraorbital patch.* This is separated from the gular patch by a white stripe.

* *Abdomen.* Immaculate in all but one specimen, which bears a few spots medially.

* *Skin texture.* The dorsum is smooth in six specimens and granular in four, mainly laterally. The ventrum is smooth.

* *Colour and markings.* The interocular bar is light brown in five specimens, off-white in two and light to dark pink in three; the paravertebral patches are light brown in four specimens, off-white in two and light to dark pink in four; the dorsolateral patches are white in five specimens, light brown in three and pink in two. The dorsum medial to the dorsolateral patches is uniform light brown in two specimens; light brown with black spots, anteriorly, in one; uniform dark brown in one and dark brown with scattered black spots in four. Lateral (ventral) to the dorsolateral patches the colour is dark brown.

3.3.2.6..Loc.34 (Mkuze) (12 specimens)

* *Tympanum.* Not distinguishable in 11 specimens, faintly visible in one.

* *Length of outer finger.* The outer finger falls just short of the distal subarticular tubercle of the fourth finger in eight specimens and reaches or barely reaches it in four.

**Proximal subarticular tubercle of 4th finger.* This is not divided.

* *Inner and outer metatarsal tubercles.* These are separated by a cleft in 11 specimens and by a groove in one.

* *Length of outer toe.* The outer toe falls just short of the basal subarticular tubercle of the fourth toe in 10 specimens and reaches it in two.

* *Light interocular bar.* This is indistinct in seven specimens and distinct in five. The bar has a black border in one specimen.

* *Light paravertebral patches.* These are distinct in seven specimens and indistinct in five. The patches of five specimens have black borders.

* *Light dorsolateral patches.* These are distinct in 11 specimens and indistinct in one.

* *Vertebral line.* Absent.

* *Heel to heel line.* Absent.

* *Urostylar patch.* Absent in eight specimens and poorly developed in four.
* *Gular region.* A uniformly dark gular patch is present in seven specimens and the patch is marbled in five.
* *Dark infraorbital patch.* This is separated from the gular patch by a white stripe in 11 specimens and the patches are joined in one.
* *Abdomen.* Immaculate white in nine specimens lightly spotted medially in three.
* *Skin texture.* The dorsum is granular to densely granular in 10 specimens, with the granulations more prominent laterally. Two specimens are smooth dorsally. The ventrum is smooth with some lateral granulations present in three specimens.
* *Colour and markings.* The interocular bar is very light brown in five specimens, off-white in four, cream in two and grey in one. The paravertebral patches are light brown in three specimens, off-white in five, cream in two, pink in one and grey in one. The dorsolateral patches are white in three specimens, cream in six, light brown in two and light pink in one. The dorsum medial to the dorsolateral patches is uniform light brown in two specimens, light brown with conspicuous black spots in four, uniform dark brown in three and dark brown with black spots in three. Lateral to the dorsolateral patches the colour is dark brown in nine specimens and light brown in three.

3.3.3 **Escarpment of KwaZulu/Natal, Swaziland, Mpumalanga and Northern Province**

On the basis of advertisement call structure, these populations are regarded as conspecific with those from the coastal plain (see Section A, 3.6) and are assigned to *mossambicus*. The same morphological characters that were treated in the previous section are examined below.

3.3.3.1 Loc.2 (Belfast) & Loc.27 (Lydenburg 1) (15 specimens; Fig.17e)

* *Tympanum.* Not distinguishable.
* *Length of outer finger.* Outer finger reaches the proximal subarticular tubercle of the fourth finger in three specimens, barely reaches it in six and falls just short in six.
* *Proximal subarticular tubercle of 4th finger.* Not divided in 14 specimens, divided

in one.

* *Inner and outer metatarsal tubercles.* These are separated by a cleft.

* *Length of outer toe.* The outer toe reaches the basal subarticular tubercle of the fourth toe in three specimens, falls just short in 10 and far short in two.

* *Light interocular bar.* This is distinct in two specimens, indistinct to very indistinct in 12 and absent in one.

* *Light paravertebral patches.* These are distinct in 11 specimens and very indistinct in four from Lydenburg. In 12 specimens the patches have black borders and in eight the posterior group of patches have a deeply notched lateral border.

* *Light dorsolateral patches.* These are distinct in six specimens, indistinct in seven and not distinguishable in two.

* *Vertebral line.* This is absent in 14 and very indistinct in one specimen.

* *Heel to heel line.* This is absent in 14 and indistinct in one specimen.

* *Urostylar patch.* Not present.

* *Gular region.* This area is uniformly dark in 10 specimens and mottled in five. In two specimens the patch is incompletely divided into two longitudinal dark bands.

* *Dark infraorbital patch.* This is separated from the gular patch by a white stripe.

* *Abdomen.* This is immaculate white in 11 specimens and lightly spotted in four.

* *Skin texture.* The dorsum is entirely smooth in eight specimens and granular laterally in seven. The ventrum is entirely smooth.

* *Colour and markings.* The interocular bar and paravertebral patches are light brown (apricot in some live specimens) to brown. The dorsolateral patches are light brown in 11 specimens, cream in three and off-white in one. The dorsum medial to the dorsolateral patches is uniform light brown in two specimens with light to very heavy black speckling and spotting in 13. Lateral (ventral) to the dorsolateral patches the colour is dark brown in eight specimens and light brown in seven.

3.3.3.2 Loc.31, 32 & 33 (Mbabane1, 2 & 3) (5 specimens)

* *Tympanum.* Not distinguishable.

* *Length of outer finger.* The outer finger barely reaches the distal subarticular tubercle of the fourth finger in three specimens and falls just short of it in two.

* *Proximal subarticular tubercle of 4th finger.* Not divided.

* *Inner and outer metatarsal tubercles.* These are separated by a cleft.

* *Length of outer toe.* . This falls just short of the basal subarticular tubercle in three specimens and far short in two.

* *Light interocular bar.* This is distinct in two specimens and very indistinct in three. In one specimen the bar has a black margin.

* *Light paravertebral patches.* These are distinct in four specimens and indistinct in one. In four specimens the patches are outlined in black.

* *Light dorsolateral patches.* Distinct.

* *Vertebral line.* Absent.

* *Heel to heel line.* Absent.

* *Urostylar patch.* Absent.

* *Gular region.* This is uniformly dark in three specimens and marbled in two. In all specimens the posterior half of the patch is divided by a median light stripe.

* *Dark infraorbital patch.* This is separated from the gular patch by a white stripe which extends from the upper jaw to the arm.

* *Abdomen.* This is lightly freckled in three specimens, heavily freckled in one and immaculate white in one.

* *Skin texture.* The dorsum is smooth in two specimens, while in three it is slightly granular laterally. The ventrum is smooth.

* *Colour and markings.* The interocular bar is light brown in four specimens and off-white in one; paravertebral patches are light brown (apricot in some live specimens) to brown in four specimens and cream in one; dorsolateral patches are cream in three specimens, off-white in one and light pink in one. The dorsum medial to the dorsolateral patches is light brown in three and dark brown in two specimens with varying degrees of dark spotting and speckling. Lateral (ventral) to the dorsolateral patches the colour is dark brown.

3.3.3.3. Loc.56 (Wakkerstroom) & Loc.43 (Piet Retief) (3 specimens)

* *Tympanum.* Not distinguishable.

* *Length of outer finger.* The outer finger reaches the distal subarticular tubercle of

the fourth finger.

* *Proximal subarticular tubercle of 4th finger.* Not divided.

* *Inner and outer metatarsal tubercles.* These are separated by a cleft.

* *Length of outer toe.* The outer toe falls just short of the basal subarticular tubercle of the fourth toe.

* *Light interocular bar.* Indistinct.

* *Light paravertebral patches.* Indistinct in two specimens, distinct in one. The patches have black margins which are deeply notched laterally.

* *Light dorsolateral patches.* Distinct.

* *Vertebral line.* Absent.

* *Heel to heel line.* Present in one specimen, but indistinct.

* *Urostylar patch.* Present in one specimen, but indistinct.

* *Gular region.* This is marbled in one Wakkerstroom specimen and uniformly dark in the other two specimens. In the latter the gular patch is divided into two parallel dark bands by a median white stripe.

* *Dark infraorbital patch.* This is separated from the gular patch by a white stripe.

* *Abdomen.* Immaculate white.

* *Skin texture.* The dorsum is granular medially in one specimen from Wakkerstroom but smooth in the other specimens. The ventrum is smooth.

* *Colour and markings.* The interocular bar and paravertebral patches are light brown, and the dorsolateral patches are off-white to white. The dorsum medial to the dorsolateral patches is light brown and heavily speckled and spotted in black. Lateral (ventral) to the dorsolateral patches the colour is light brown in two specimens and dark brown in one.

3.3.3.4 Loc.8 (Graskop 1) (18 specimens; Fig.17f, 30a-d)

* *Tympanum.* Not distinguishable in 17 specimens and faintly visible in one.

* *Length of outer finger.* The outer finger falls far short of the distal subarticular tubercle of the fourth finger in six specimens, just short in eight, barely reaches the tubercle in three and reaches it in one.

* *Proximal subarticular tubercle of 4th finger.* Not divided in 15 specimens and di-

vided in three.

* *Inner and outer metatarsal tubercles*. These are separated by a cleft.

* *Length of outer toe*. The outer toe falls just short of the basal subarticular tubercle of the fourth toe in 12 specimens, and far short of it in six.

* *Light interocular bar*. This is distinct in eight specimens, indistinct in four and absent in six.

* *Light paravertebral patches*. These are distinct in 16 specimens, indistinct in one and absent in one. In 12 specimens the patches have black margins and in eight the margins are deeply notched laterally.

* *Light dorsolateral patches*. These are distinct in 14 specimens, indistinct in three and absent in one.

* *Vertebral line*. This is absent in 12 specimens, indistinct in five and distinct in one. It is present posteriorly in three specimens and extends to the head in three.

* *Heel to heel line*. Absent in 12 specimens, indistinct in three and distinct in three.

* *Urostylar patch*. Absent in 13 specimens, indistinct in four and distinct in one.

* *Gular region*. The gular patch is uniformly dark in 11 specimens and marbled in seven. The patch is completely divided into two dark bands by a light median stripe in three specimens, and partly divided in three.

* *Dark infraorbital patch*. This is separated from the gular patch by a white stripe in 13 specimens and continuous with the gular patch in five.

* *Abdomen*. This is immaculate white in 14 specimens and lightly freckled in four.

* *Skin texture*. The dorsum is granular in 13 specimens and smooth in five. The ventrum is smooth in 16 specimens and granular laterally in two.

* *Colour and markings*. Very variable (Fig.30a-d). The interocular bar is light brown in 11 specimens and off-white in one. The paravertebral patches are light brown in 11 specimens (apricot in some live specimens) brown in one and off-white in five. The dorsolateral patches are light brown in seven specimens, brown in one, cream in one, white in five and off-white in two. The dorsum medial to the dorsolateral patches is dark brown to almost black with dark speckles and spots in 14 specimens, light brown (apricot in life) with dark speckles and spots in three and uniform dark brown in one. The area lateral (ventral) to the dorsolateral patches is dark brown to black.

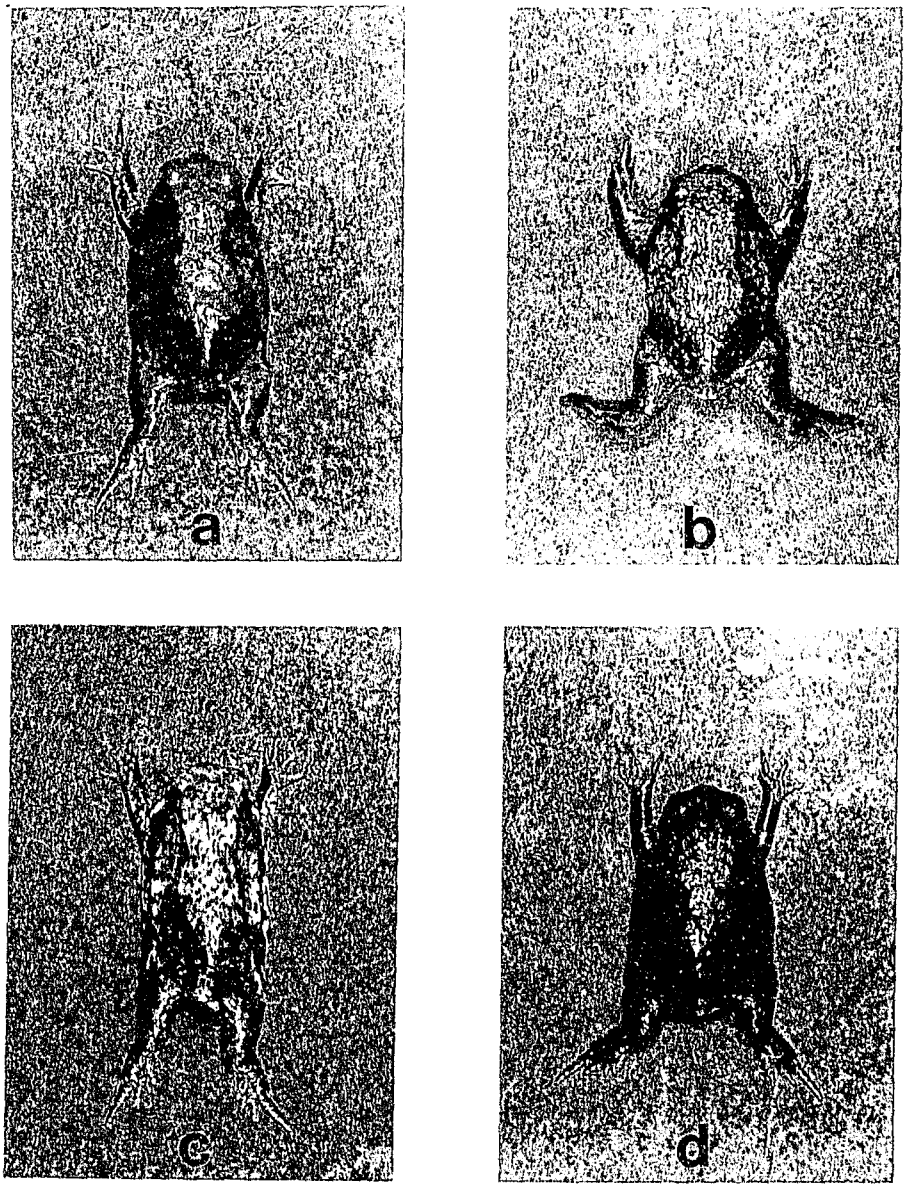

FIGURE 30. Variation in dorsal colouration of *B. mossambicus* males from Loc.8 (Graskop 1).

3.3.3.5 Loc.11 (Haenertsburg 2) (3 specimens)

* *Tympanum.* Not distinguishable in two specimens, very faintly discernible in one.
* *Length of outer finger.* The outer finger barely reaches the distal subarticular tubercle of the fourth finger in two specimens and reaches it in one.
* *Proximal subarticular tubercle of 4th finger.* Not divided.
* *Inner and outer metatarsal tubercles.* These are separated by a cleft in two specimens and by a groove in one.
* *Length of outer toe.* The outer toe falls just short of the basal subarticular tubercle of the fourth toe in two specimens and far short of it in one.
* *Light interocular bar.* Present but indistinct.
* *Light paravertebral patches.* Indistinct in two specimens and distinct in one.
* *Light dorsolateral patches.* Distinct.
* *Vertebral line.* Absent.
* *Heel to heel line.* Absent.
* *Urostylar patch.* Absent.
* *Gular region.* The patch is uniformly dark in two specimens and spotted in one.
* *Dark infraorbital patch.* The patch is separated from the gular patch by a light stripe in one specimen and tenuously separated in two.
* *Abdomen.* Immaculate white.
* *Skin texture.* The dorsum is granular, but smooth medially in one specimen. The ventrum is smooth, but granular laterally in one specimen.
* *Colour and markings.* The interocular bar, paravertebral patches and dorsolateral patches are cream to light brown. The dorsum medial to the dorsolateral patches is light brown with dark speckles and spots in two specimens, and dark brown with dark speckles and spots in one. Lateral (ventral) to the dorsolateral patches the colour is light brown in two specimens and dark brown in one.

3.4 Synopsis

3.4.1 Diagnostic characters (Poynton & Broadley 1985)

Variation in morphological characters observed in the three major groups of populations assigned to *mossambicus*, viz: northern Mozambique, the coastal plain of KwaZulu/Natal and the eastern escarpment, is summarised here. The extent to which the morphology of these samples conforms to the diagnosis for *mossambicus* is discussed.

* *Outer finger reaching or nearly reaching distal subarticular tubercle of fourth finger.*

The outer fingers of 143 specimens "reach or nearly reach the distal subarticular tubercle of the fourth finger", and thus conform to the diagnostic character state for *mossambicus*. However, 23 (14%) specimens from populations in coastal KwaZulu/Natal and Graskop, have shorter outer fingers which fall far short of the distal subarticular tubercle, and thus conform to the diagnostic character state for *adspersus*.

* *Inner and outer metatarsal tubercles separated by a cleft.*

The tubercles are separated by a cleft in 143 specimens, a groove in 20 and are united in three. Most specimens therefore conform to the diagnostic character state for *mossambicus*, but some variation (14%) is present.

* *No light paravertebral or dorsolateral patches.*

The northern Mozambique samples generally conform to this character state although weakly developed dorsolateral patches are present in three specimens from the Island and mainland.

However, the southern samples are quite different: paravertebral patches are distinct in 92, indistinct in 26 and absent in two specimens, while dorsolateral patches are distinct in 80, indistinct in 32 and absent in three specimens. The samples

from the coast and the escarpment are very similar in terms of the position and shape of the patches and the presence of dark, notched borders. These samples conform to the character state currently considered diagnostic of *adspersus*.

** Gular region with a single uniformly darkened to (less usually) marbled to freckled patch.*

This character is sometimes difficult to score because of the intergradation between the two character states. In some specimens the marbled or freckled pattern is obscured by darkening of the area between the black markings: as this dark "overlay" becomes darker the mottled pattern eventually becomes invisible.

Another problem is that this process of darkening seems to affect the anterior regions first so that some individuals possess a patch which is uniformly darkened anteriorly and marbled posteriorly. This is the case in the Island sample, where the gular patch is uniformly darkened anteriorly and freckled posteriorly in 13 specimens, and completely freckled in seven. In the mainland specimens (northern Mozambique), which are generally much darker, the patch is uniformly darkened. In the southern samples, the gular patch is uniformly darkened in 87 specimens, mottled in 32 and immaculate white in one. Because of its variability, this character is not considered to have diagnostic value.

A variation present in 49 specimens (41%) from the south, which is absent in specimens from northern Mozambique, is an immaculate white midventral band which divides the patch into two parallel dark bands or patches (an *adspersus* character). This white band varies in extent and is sometimes only present posteriorly. The variability of this character severely limits its diagnostic value.

** Dark infraorbital patch extending to base of arm, in mainland material usually broadly continuous with gular patch.*

In the sample from northern Mozambique the two patches are separated by a white stripe in most (19) Island specimens while the opposite holds true for the mainland specimens where the patches are fused in most (18) specimens. In the southern sam-

ples the patches are separated in 99 specimens, completely fused in six, and tenuously connected in 15. There is a tendency for the white stripe, which separates the patches, to beome less obvious or disappear in heavily pigmented individuals.

Since the advertisement call data confirms that the Island and mainland populations in northern Mozambique are conspecific, and considering the variation of these populations in respect of this character, it seems to have no diagnostic value at the species level.

3.4.2 Other characters

* *Tympanum.*

This structure is not distinguishable in the vast majority of specimen.Upon close examination its faint outline can be seen in five specimens. This is a stable character and no significant variation between groups is present.

* *Proximal subarticular tubercle of 4th finger.*

This tubercle is undivided in 156 specimens, divided in five, and divided on only one hand in five specimens. This is a stable character and no significant variation between population groups is present.

* *Length of outer toe.*

The outer toe falls far short of the basal tubercle of the fourth toe in 43 specimens, just short of it in 118, and reaches the tubercle in five. While it is easy to judge whether or not the outer toe reaches or falls short of the basal tubercle of the fourth toe (only 3% reach it), it is sometimes difficult to decide whether the toe falls "just short" or "far short" of the tubercle, without resorting to actual measurement under the microscope. Thus a greater margin of error is expected in the latter judgement. The former character state ("reaches") probably incorporates less of the range of variation in the position of the outer toe, than do the other two ("falls just short of" and "falls far short of"). The latter measures do not reveal any significant dichotomy in the samples and are therefore of little taxonomic value.

A greater variation is seen in the southern population groups than in the northern sample. Perhaps this is due to the fact that the northern sample represents only three populations whereas the southern samples represent 20 populations.

* *Light interocular bar.*

The interocular bar is visible in 136 specimens and not distinguishable in 30 (18%). This character is too variable for it to be of any diagnostic value, and may become indistinct or disappear as the colour changes with preservation,. It is, in any case, common to most *Breviceps* species.

* *Vertebral line.*

A vertebral line is present but indistinct in most (16) Island specimens and absent in most (19) mainland specimens from northern Mozambique. In the southern samples it is best developed in the coastal populations from northern KwaZulu/Natal and less common in the specimens from the escarpment. In total, this character is present in 78 specimens and absent in 88. Where present it is often indistinct. In most specimens it is confined to the posterior dorsum but in some it extends the the head. Because of its variability, this character has no diagnostic value.

* *Heel to heel line.*

This character is present in all the major groups of populations. It is distinct in 21 specimens, indistinct in 53 and absent in 92. Because of its variability, this character is of no diagnostic value.

* *Urostylar patch.*

This character is entirely absent from the northern Mozambique sample but occurs in a few populations to the south, notably Eshowe (Fig.18d) and Craskop. A distinct light patch over the urostyle is present in four specimens and indistinct in 11. This character occurs in equally low numbers in the *adspersus* sample, but its mere presence affects the diagnostic value of this character in respect of *B. poweri.*

* *Abdomen.*

The abdomen is immaculate white in 126 specimens and freckled to varying degrees in 40. Freckling is more common in some populations than in others, eg. Mtunzini, Eshowe and Mbabane, and is entirely absent in the Mozambique Island and St Lucia populations. Because of its variability, this character has no diagnostic value.

* *Skin texture.*

The dorsum is smooth in 104 specimens and granular in 62, usually laterally. The ventrum is smooth in all except six specimens which are granular laterally. Only two specimens from northern Mozambique have a slightly granular dorsum while this character is more common in southern populations such as Mtunzini, Mkuze, Belfast and Graskop. Because of its variability, this character has no diagnostic value.

* *Colour and markings.* (Figs.3, 18, 29 & 30)

Crypsis is obviously of great value to slow-moving and relatively defenceless animals such as these, particularly when feeding at the surface during the day, or concealing themselves at their call sites. Given the wide variety of soil and vegetation types in the areas that they inhabit, it is not surprising that colour and markings are highly variable among and within the populations sampled.

Thus the population inhabiting leaf litter on the light brown sandy soil of Mozambique Island (Fig.31a) are as perfectly camouflaged as the much darker populations that live on the dark, loamy soils of Nampula (Fig.3c), Mtunzini (Fig.31b), Eshowe and Graskop (Fig.30). Similarly, populations which occur on the coastal plain north of St Lucia, display a variety of colours, including white and red (Fig.29), on a grey, cream or tan background, which match the light, almost white sand and variegated leaf litter that occur in their habitat.

Black spots, speckles and stripes break up outlines, conceal the eye (Fig.31b) and function in mimesis, eg. the vertebral line and black spots on specimens from Mozambique Island increase their resemblance to the decaying leaves in their environment (Fig.31a). Thus colouration and markings have no diagnostic value in this species.

FIGURE 31. Crypsis and mimesis in populations of *B. mossambicus* from (a) Loc.36 (Mozambique Island) and (b) Loc.37 (Mtunzini).

4. *Breviceps verrucosus* Rapp 1842

4.1 Taxonomic status

On the basis of the material available to him, Poynton (1964) changed the status of *B. tympanifer* Hewitt, to *B. verrucosus tympanifer* Hewitt. The subspecies *tympanifer* was thought to be restricted to the Eastern Cape, south of the Kei River, while *verrucosus* was distributed northwards through KwaZulu/Natal and along the eastern escarpment into Mpumalanga.

However, Lambiris (1989) noted the presence of *tympanifer* in Hillcrest, KwaZulu/Natal, and the present study reveals a population near Lydenburg, Mpumalanga and populations at Harding and Eshowe which exhibit combinations of characters diagnostic of both subspecies.

This study shows that no significant inter-population differences in advertisement call structure exist: this supports Poynton's view that *verrucosus* and *tympanifer* are conspecific. The difference in markings is regarded as a consequence of crypsis, operating in different habitats: grassland in the case of *tympanifer* and forest in the case of *verrucosus* (see 4.2 below).

The recognition of two geographically isolated subspecies is no longer tenable.

4.2 Material examined

Thirty-one males from 6 populations (listed below) were examined.

Loc. 45 (Seymour, E. Cape)	2 males	*tympanifer*	grassland
Loc. 54 (Stutterheim)	9 males	*tympanifer*	grassland
Loc. 12 (Harding)	9 males	*? verrucosus*	forest
Loc. 6 (Eshowe)	3 males	*? verrucosus*	cultivated
Loc. 27 (Lydenburg 1)	5 males	*tympanifer*	grassland
Loc. 8 (Graskop 1)	3 males	*verrucosus*	forest fringe

4.3 **Diagnostic characters** (Poynton, 1964, pp.71-72) (Fig.4a-d; 27c-d; 32a-d)

* *Tympanum usually visible, but sometimes concealed by granules.*

The tympanum is distinguishable in all specimens as a smooth, slightly depressed, oval area of skin posterior and inferior to the eye. It is indistinct in one specimen from Eshowe. Most of the Stutterheim, Harding and Lydenburg specimens bear a few small tubercles on the tympanum, usually near the perimeter. These tubercles are smaller than those occuring on the skin adjacent to the tympanum. All specimens thus conform to the diagnosis in respect of this character.

* *Outer toe extending well beyond basal tubercle of fourth, reaching or extending beyond second.*

It is assumed that "basal tubercle" refers to the proximal subarticular tubercle, and that by "second", Poynton means second subarticular tubercle of the fourth toe. However,Lambiris (1989, pp.61-62), in his diagnosis for *B.v. verrucosus*, states: "Outer toe extends well beyond basal tubercle of fourth, and at least reaches that of the second toe." It is not clear why he chooses to use the basal tubercle of the second toe as a reference point, since the tip of the outer toe lies far beyond the level of the basal tubercle of the second toe: in fact it extends beyond the end of the second toe! There is therefore little likelihood that the outer toe would not "at least" reach the basal tubercle of the second toe.

The outer toe reaches the proximal subarticular tubercle of the fourth toe in four specimens (from Harding) and extends beyond it in 27, but never reaches the level of the second subarticular tubercle of the fourth toe. Thus the outer toe in these specimens is not quite as long as is suggested in Poynton's diagnosis.

* *Dorsal surface densely granular; granules large and usually deeply pitted.*

This character is present in all specimens except six (from various localities) in which the area of skin lying between the glandular ridges is smooth or only slightly granular. The granules are larger than those of *adspersus* and bear a single, deep opening at the apex rather than 5-6 small openings.

* *Usually one, two or more pairs of longitudinal glandular ridges on back.*
Glandular ridges are distinct in nine specimens (Figs.4a, 32c&d), indistinct in 14 and absent in seven. In 10 specimens more than one pair of ridges are present, but the inner pairs are shorter and less distinct. The ridges are formed by the fusion of adjacent tubercles in an anterior-posterior direction. In 14 specimens the ridges converge slightly in the scapular region and diverge anterior and posterior to this area. When present, this character has diagnostic value in that it distinguishes *verrucosus* and *sylvestris* from the other species dealt with in this study.

* *Ventral surface densely granular.*
This character state is present in all specimens.

* *Uniform dark to light brown above, with longitudinal glandular ridges usually coloured more darkly in younger specimens (verrucosus pattern),* **or** *overall dorsal colour brown, but a light dorsal band, about the width of the head, usually present. Sometimes upper surface marked with an intermingling of brown and yellow, with yellow predominating in the mid-dorsal area (tympanifer pattern).*
The Seymour, Stutterheim and Lydenburg specimens, all collected in grassland habitats, display the *tympanifer* pattern of a lighter, mottled mid-dorsal band and darker mottling laterally (Figs.4c, 32a,b&d).

The Harding specimens, collected in closed canopy forest, are uniformly black (Fig.27d), with the exception of two specimens which show slight mottling laterally and one specimen (Fig.27c) in which a mottled mid-dorsal band shows through the darker pigmentation. Thus, in these specimens, the *tympanifer* pattern appears to be masked by darker pigmentation.

The Graskop specimens, collected on the fringes of forest patches are black with numerous, small, yellow or cream speckles (Fig.4a). These, together with the Harding specimens, most closely fit the *verrucosus* pattern.

The Eshowe specimens are predominantly light brown above with a few scattered